December 18, 2001

Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency
P. O. Box 1473
Merrifield, VA 22116

RE: Olefins Panel Test Plan for Fuel Oils Category, HPV Registration No.

Dear Ms. Whitman:

The Olefins Panel of the American Chemistry Council submits its test plan for the Fuel Oils Category under the High Production Volume (HPV) Challenge Program. The CAS numbers in this category are listed in the attached table. This category includes CAS numbers that represent streams with a carbon number distribution that ranges predominantly from C8 and higher. The plan addresses the category by evaluating data available for the CAS numbers in the category and for products not in this category whose data can be used to read across to products in the category. Supporting data from key components will also be used. No additional testing will be conducted for potential human health effects, but aquatic toxicity and biodegradation tests are proposed for some streams.

In preparing this test plan, the Panel has given careful consideration to the principles contained in the letter EPA sent to all HPV Challenge Program participants on October 14, 1999. As requested by EPA in that letter, the Panel has sought to maximize the use of scientifically appropriate categories of related chemicals and of structure activity relationships. The Panel has coordinated with other industry groups covering related chemicals. Additionally, and also as requested in EPA's letter, in analyzing the adequacy of existing data, the Panel has conducted a thoughtful, qualitative analysis rather than a rote checklist approach. The Panel has taken the same thoughtful approach when developing this test plan and believes it conforms to those principles.

If you have any questions, please contact Elizabeth Moran, Manager of the Olefins Panel at (301) 924-2006 or Elizabeth_Moran@americanchemistry.com.

Courtney M. Price
Vice President, CHEMSTAR

cc: C. Auer, EPA
B. Leczynski, EPA
S. Russell, ACC
J. Keith, ACC

CAS Numbers used in the Fuel Oils Category

CAS #	CAS Inventory Name
64741-62-4	Clarified oils, petroleum, catalytic cracked
64742-90-1	Residues, petroleum, steam-cracked
68131-05-5	Hydrocarbon oils, process blends
68409-73-4	Aromatic hydrocarbons, biphenyl-rich
68475-80-9	Distillates, petroleum, light steam-cracked naphtha
68513-69-9	Residues, petroleum, steam-cracked light
68514-34-1	Hydrocarbons, C9-14, ethylene-manuf.-by-product
68527-18-4	Gas oils, petroleum, steam-cracked
68921-67-5	Hydrocarbons, ethylene-manuf.-by-product distn. residues
69013-21-4	Fuel oil, pyrolysis
69430-33-7	Hydrocarbons, C6-30
8002-05-9	Petroleum

Note: The definitions, found in the TSCA Chemical Substance Inventory, for the CAS numbers included in this group are vague with respect to composition. Therefore, it is not uncommon to find that the same CAS number is correctly used to describe different streams (compositions) or that two or more different CAS numbers are used to describe the same stream (composition)

AR201-13435A

High Production Volume (HPV) Chemical Challenge Program

TEST PLAN

For The

Fuel Oils Category

Prepared by:

American Chemistry Council
Olefins Panel
HPV Implementation Task Group

December 18, 2001

PLAIN ENGLISH SUMMARY

This test plan addresses streams that are products of the ethylene process and are used as fuel oils. The Fuel Oils Category includes CAS numbers that represent streams with a carbon number distribution that ranges predominantly from C8 and higher. The plan addresses the category by evaluating the data available for representative streams, as well as for chemicals and products not in this category whose data can be used to read across to products in this category. Supporting data on key chemical components, such as naphthalene and biphenyl, will be reviewed as part of other test plans under the EPA High Production Volume (HPV) Challenge Program, the ICCA (International Council of Chemical Associations) program, or from chemicals already sponsored in the European Union Existing Substances Risk Assessment program or the OECD (Organization for Economic Cooperation and Development) SIDS (Screening Information Data Sets) program. No additional toxicity testing for potential human health effects is necessary. However, aquatic toxicity and biodegradation tests will be conducted to fully characterize these streams. In addition, data and/or technical discussions will be prepared for the remaining fate endpoints, and a comprehensive physicochemical database will be developed that contains measured and calculated data.

EXECUTIVE SUMMARY

The Olefins Panel (Panel) of the American Chemistry Council and the Panel's member companies hereby submit for review and public comment the Fuel Oils Category test plan under the Environmental Protection Agency's (EPA) High Production Volume (HPV) Chemical Challenge Program. It is the intent of the Panel and its member companies to use new information in conjunction with a variety of existing data and scientific judgment/analysis to adequately characterize the SIDS (Screening Information Data Set) human health, environmental fate and effects, and physicochemical endpoints for this category.

This test plan addresses streams that are products of the ethylene process that contain mostly cyclic olefins and aromatic hydrocarbons that are generally carbon number 8 and higher, with some lighter components. The streams are similar in that they are all complex streams (containing several different chemicals) that generally consist of the same higher-boiling hydrocarbons, but at varying concentrations. These streams are represented using 12 CAS numbers. The streams are frequently utilized in the fuel oil market and sometimes for chemical purposes. This test plan addresses the category by evaluating data on select mixed process streams that represent typical fuel oil products. Robust summaries are provided for the following:

- Light Pyrolysis Fuel Oil
- Aromatic Pyrolysis Oil and Rerun Tower Bottoms
- Biphenyl Feedstock
- Coal Derived Fuel Oils

Results from studies on these streams will be used to read across to evaluate other members of the Fuel Oils Category. Additional supporting data exist, or will be collected on many of the components of the streams in this category as part of other test plans under the HPV program, the ICCA (International Council of Chemical Associations) program, or from chemicals already sponsored in the OECD (Organization for Economic Cooperation and Development) SIDS (Screening Information Data Sets) program or European Union Existing Substances Risk Assessment program.

Predictive computer models will be used to develop relevant environmental fate and physicochemical data for chemicals in products of the Fuel Oils Category. Environmental fate information will be summarized either through the use of computer models when meaningful projections can be developed or in technical discussions when computer modeling is not applicable. For mixed streams, physicochemical properties will be represented as a range of values according to component composition. These data will be calculated using a computer model cited in an EPA guidance document prepared for the HPV Challenge Program.

In preparing this test plan, the Panel has given careful consideration to the principles contained in the letter EPA sent to all HPV Challenge Program participants on October 14,

1999. As directed by EPA in that letter, the Panel has sought to maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships. Additionally, and also as directed in EPA's letter, in analyzing the adequacy of existing data, the Panel has conducted a thoughtful, qualitative analysis rather than a rote checklist approach. The Panel has taken the same thoughtful approach when developing its test plan. The Panel believes its test plan conforms to the principles articulated in EPA's letter.

After careful evaluation of the existing data, no additional toxicity testing for potential human health effects is proposed. However, aquatic toxicity and biodegradation tests are proposed to characterize the potential toxicity of these streams to the environment within the HPV program. In addition, data and/or technical discussions will be prepared for the remaining fate endpoints, and a comprehensive physicochemical database will be developed that contains measured and calculated data.

LIST OF MEMBER COMPANIES
THE OLEFINS PANEL

The Olefins Panel includes the following member companies:

ATOFINA Petrochemicals, Inc.*
BP Chemical Company*
Chevron Phillips Chemical Company LP
The Dow Chemical Company
E.I.du Pont de Nemours and Company*
Eastman Chemical Company
Equistar Chemicals, LP
ExxonMobil Chemical Company
Formosa Plastics Corporation, U.S.A.
The Goodyear Tire & Rubber Company*
Huntsman Corporation
Koch Industries*
NOVA Chemicals Inc.*
Noveon, Inc.*
Sasol America, Inc.*
Shell Chemical Company*
Sunoco, Inc.*
Texas Petrochemicals Corporation*
Westlake Chemical Corporation*
Williams Olefins, LLC*

*These companies are part of the Olefins Panel but do not produce streams in the Fuel Oils Category

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TEST PLAN FOR THE FUEL OILS CATEGORY

I. INTRODUCTION

The Olefins Panel (Panel) of the American Chemistry Council and the Panel's member companies have committed to develop screening level human health effects, environmental effects and fate, and physicochemical data for the Fuel Oils Category under the Environmental Protection Agency's (EPA's) High Production Volume (HPV) Challenge Program (Program).

In preparing this test plan, the Panel has given careful consideration to the principles contained in the letter EPA sent to all HPV Challenge Program participants on October 14, 1999. As requested by EPA in that letter, the Panel has sought to maximize the use of scientifically appropriate categories of related chemicals and of structure activity relationships. The Panel has coordinated with other industry groups covering related chemicals. Additionally, and also as requested in EPA's letter, in analyzing the adequacy of existing data, the Panel has conducted a thoughtful, qualitative analysis rather than a rote checklist approach. The Panel has taken the same thoughtful approach when developing this revised test plan and believes it conforms to those principles.

This plan identifies CAS numbers used to describe process streams in the category, identifies existing data of adequate quality for products included in the category, and outlines testing needed to develop screening level data for this category under the Program. This document also provides the testing rationale for the Fuel Oils Category. The objective of this effort is to identify and develop sufficient test data and/or other information to characterize the human and environmental health and environmental fate for the category in compliance with the EPA HPV Program. Physicochemical data that are requested in this program will be developed and calculated as described in the EPA guidance documents.

II. DESCRIPTION FOR THE FUEL OILS CATEGORY

A. The Category

The Fuel Oils Category was developed by grouping 8 ethylene industry streams made up of hydrocarbons that are generally carbon number 8 (i.e. C8) and higher with varying amounts of lower boiling materials. The streams are similar in that they are all complex streams that consist predominantly of the same higher-boiling hydrocarbons, mostly cyclic olefins and aromatics, but at varying concentrations. The Panel believes these streams are similar from both a process and a toxicology perspective, which is why this group is considered a category for purposes of the HPV Program. Twelve CAS numbers (Table 1) are used to describe the 8 process streams (Table 2) arising from the ethylene process that are commercial products or isolated intermediates. A process stream is a mixture of chemicals that arises from a chemical reaction or separation activity. The CAS numbers used to represent these mixed streams are generally vague with respect to the specifics that distinguish the streams within

the category. Therefore, more than one CAS number may correctly represent a single stream and a CAS number may be applicable to more than one stream. A description of the ethylene and associated stream production processes is included in Appendix 1.

The streams in this category consist of complex hydrocarbon reaction products with a carbon number distribution that is predominantly in a C8 and higher range. The 1,3-butadiene content is negligible. The typical compositions of the streams in this category are shown in Table 3. Descriptions of the eight streams in the Fuel Oils Category are presented below.

B. Fuel Oils Streams

(1) Heavy Pyrolysis Fuel Oil from the Ethylene Process Unit: In ethylene plants cracking liquid feedstocks, the cracking furnace effluent (after heat recovery) is further quenched by injection of recycled quench oil. This step results in the condensation of higher boiling hydrocarbon compounds that are typically separated from the rest of the furnace effluent as the bottoms of the primary fractionation tower or oil quench tower. Lights are stripped from the excess oils generated from this quench system, resulting in the stream identified here as heavy pyrolysis fuel oil consisting of C10+ and considerable PAHs.

(2) Light Pyrolysis Fuel Oil from the Ethylene Process Unit: In some cases, a light pyrolysis fuel oil is produced from the oil quench system in an ethylene plant that cracks liquid feedstocks. This stream may be produced as a side draw from the primary fractionation tower. The stream typically has a carbon number distribution of C9 to C14 and the major components are naphthalene (30 to 60%), methyl naphthalenes and other substituted one and two ring aromatics.

(3) Quench Oil from the Ethylene Process Unit Water Quench System: In ethylene plants cracking only gases, the cracking furnace effluent (after heat recover) may be further quenched with water. This step results in the condensation of a relatively small amount of higher boiling hydrocarbon components that, after stripping to remove lights, may be isolated as the Quench Oils from of the Ethylene Process Unit water quench system. This stream is predominant C7 through components boiling at 650°F or higher. The reported composition indicates 0.1 % benzene, 5% toluene, 12% C8 aromatics, 5% naphthalene, 10% anthracene and 65% C7-C18 cyclic olefins.

(4) Pyrolysis Fuel Oil from Pyrolysis Gasoline Distillation: This stream is separated by distillation from pyrolysis gasoline, as a bottoms product. The reported composition indicates a carbon number distribution of from C9 to hydrocarbons boiling at 650°F or higher. The reported typical composition includes 20% dicyclopentadiene, 30% codimers of C5 and C6 monomers, 20% naphthalene and substituted naphthalenes.

(5) Combined Fuel Oil of the Ethylene Process and Pyrolysis Gasoline Units: A single combined fuel oil stream for the ethylene process unit and the pyrolysis gasoline unit is not an uncommon situation for the industry. The carbon number distribution for this stream is generally C10 to compounds with a boiling point of 650°F or higher. At least in some cases, lower carbon number components are reported for the stream, e.g. C5s at approximately 2%

and benzene at up to 4%. The major components reported in the stream are typically 25% C9 compounds, 10-47% naphthalene and 4-30% methylnaphthalenes.

(6) Combined Fuel Oil from Benzene Hydrodealkylation (HDA) and Pyrolysis Fuel Oils: Ethylene process operations that include both a pyrolysis gasoline distillation unit and a benzene hydrodealkylation unit may combine the fuel oil streams from these two units resulting in a single isolated product. Fuel oil is produced in the benzene HDA process by the HDA reactors and separated as a distillation bottoms product. The carbon number distribution for this combined fuel stream is C9 through hydrocarbons with a boiling point of 650°F or higher, although relatively low levels of lower carbon number hydrocarbons may be present, e.g. 0.2% benzene. The major components reported in the stream include 11% C9 aromatics to naphthalene, 7.5-12% DCPD, 7-13% naphthalene, 22% methylnaphthalenes, and 25-35% biphenyl.

(7) Hydrotreated Flux Oil: This is a hydrotreated fuel oil stream with a carbon number distribution predominantly C10 to hydrocarbons with a boiling point of 650°F or higher. The stream may be produced as distillation bottoms from a pyrolysis gasoline hydrotreator unit. The components in the stream are predominantly aromatics, paraffins and cyclic compounds. This stream differs from the other fuel oils describe above in that its diolefin and vinyl aromatic content is very low.

(8) Biphenyl Concentrate: Biphenyl concentrate is a coproduct of the benzene hydrodealkylation unit that is isolated by distillation from the HDA reactor effluent. The carbon number distribution for the stream is C7 to C18, with the major component reported to be 65 to 95% biphenyl.

III. TEST PLAN RATIONALE

A. Mammalian/Human Health Effects and Test Strategy

The Fuel Oils Category consists of mixed hydrocarbon streams with a carbon number distribution that is predominantly C8 and higher. The toxic effects are dependent on the chemical composition and can be discussed as those attributed to the ≤C9 fraction, the C10 to C12 fraction, and the >C12 components. A number of the components of the streams listed in this category (see Table 3) are already SIDS (Screening Information Data Set) listed materials. Some of the remaining components will be tested as part of other test categories or by other groups within the HPV or ICCA programs. The toxicity of the ≤C9 components is similar to the hydrocarbon solvent materials being covered under the International Hydrocarbon Solvents Consortium HPV Program, and will not be further discussed in this test plan. Two of the C10-C12 materials are SIDS chemicals, naphthalene and dicyclopentadiene, and the C12 biphenyl has been volunteered for the HPV Challenge program by SOCMA (Synthetic Organic Chemical Manufacturers Association). Additionally, several component materials are undergoing risk assessment in Europe (i.e., toluene and naphthalene). Because biphenyl may be present in the Fuel Oils at

concentrations up to 95%, naphthalene at >50%, and DCPD up to 20%, the toxicity of these component materials is summarized below.

Biphenyl

The health hazards of biphenyl have been reviewed (EPA, 1984). Biphenyl is not particularly toxic by ingestion; the oral LD_{50} for the rat is 3280 mg/kg (Deichmann *et al.*, 1947), 1900 mg/kg for the mouse (Isshiki *et al.*, 1983), and for the rabbit, it is 2400 mg/kg. (Deichmann *et al.*, 1947) Intradermal injections in guinea pigs produced local necrosis and some evidence of an allergenic response. (Haley *et al.*, 1959) Repeated application of a 25% solution in olive oil to rabbit skin at 0.5 g/kg/day, 5 days/week, caused no local irritation, but these treatments did result in the death of one rabbit after 8 applications and weight loss in three others after 20 applications. (Deichmann *et al.*, 1947)

Repeated inhalation exposure by rats of biphenyl dust, impregnated on diatomaceous earth, caused irritation of the nasal mucosa, labored breathing with bronchopulmonary lesions, and slight toxic effects on liver and kidneys at a concentration of 300 mg/m^3, 7 hours/day for 64 days. Five rats died between the 29th and 49th exposure days. Rabbits were unaffected, but mice exposed at 5 mg/m^3 for this period showed signs of respiratory difficulty. Rats at this concentration were not affected. (Deichmann *et al.*, 1947)

Bionetics Research Labs (BRL, 1968) treated B6AKF1 and B6C3F1 mice (18/sex/strain) with daily gavage doses of 215 mg/kg 1,1- biphenyl in gelatin from days 7 to 28 of age. The mice were then given a diet containing 517 ppm 1,1-biphenyl for the subsequent 18 months. Positive, negative and vehicle controls were included in the study. The animals were weighed every 6 months and histopathologic examination of the lymphatic and pulmonary systems, liver, skin, mammary glands and uterus were performed at the end of the study. No increase in the incidence of tumors was found in any treated group compared with negative and vehicle controls.

Ambrose et al. (1960) fed 15 weanling albino rats/sex/group diets containing 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, or 1% (0, 0.5, 2.5, 5.0, 25, 50, 250, or 500 mg/kg/day) 1,1-biphenyl for 700 days. At the end of the treatment period, histopathologic examination of the mammary gland, pituitary, adrenals, uterus, lungs, bladder, and other tissues was performed. All rats in the 250 and 500 mg/kg/day groups showed evidence of kidney damage, including irregular scarring, lymphocytic infiltration, tubular atrophy and patchy tubular dilation. Decreased food intake, retarded growth and reduced hemoglobin levels were also observed in these groups. Survival appeared reduced in males receiving 250 mg/kg/day and in both sexes at 500 mg/kg/day, although a statistical analysis of survival was not performed. Based on these data it appears that an MTD was achieved. Although not designed as an oncology study, several malignant and benign tumors were found in both the treated and control rats. These were not considered to be related to 1,1- biphenyl treatment. A supporting unpublished study by Stanford Research Institute (SRI, 1960) was cited in which a NOAEL of 0.1% biphenyl in the diet was found in both a subchronic rat feeding study and a three- generation rat reproduction study. A NOAEL of 0.1% of diet is chosen because of the uncertainty of the significance of the effects observed at lower doses as compared to the more certain AEL of

0.5% of diet. The observation of the same NOAEL in a supporting study is also a contributing factor.

SRI (1953) fed diets containing corn oil and 0, 0.01, 0.1, or 1.0% 1,1-biphenyl to groups of 12 male and 12 female Sprague- Dawley rats for 2 years. Malignancies were reported in two rats; one (sex not specified) on the 0.1% diet had an adenocarcinoma of the colon and one female on the 1.0% diet had a peritoneal tumor. Many of the treated and control male rats had tubular dilation of the kidneys to varying degrees of severity; however, the incidence and severity of the renal lesions were greater in the group fed 1.0% 1,1-biphenyl than in the controls. This study is limited by small group sizes, excess mortality due to a refractory fulminating respiratory infection, and concurrent antibiotic therapy.

BRL (1968) treated B6AKF1 and B6C3F1 28-day-old mice (18/sex/strain) with a single subcutaneous 46.4 mg/kg dose of 1,1-biphenyl in DMSO. The mice were observed for 18 months before sacrifice. Gross and microscopic observations were performed on internal body organs of the chest and abdomen. No increase in the incidence of tumors was found in any treated group compared with controls.

Kurata et al. (1986) provided groups of 25 male F344 rats with 0.05% N- butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in the drinking water for 4 weeks followed by either a basal diet or diet containing 0.5% 1,1-biphenyl for 32 weeks. A group of five rats received the 1,1-biphenyl-containing diet without pretreatment with BBN. Rats receiving the 1,1-biphenyl diet, both with and without BBN pretreatment, gained less weight than control rats that received only BBN. In the 18 surviving rats treated with BBN followed by 1,1-biphenyl, the incidences of hyperplasia, papillomas and carcinomas in the urinary bladder was 94, 83 and 61%, respectively. These increases were statistically significant. The incidences of hyperplasia, papillomas and carcinomas in rats treated with BBN alone were 25, 12 and 0%, respectively. Hyperplasia, papillomas and carcinomas were not observed in the 5 rats fed the 1,1- biphenyl-containing diet without pretreatment with BBN. Urinary bladder stones occurred in 25% of rats receiving BBN and 1,1-biphenyl but in only 12% of the BBN control. 1,1-Biphenyl appeared to be a tumor promoter in this experiment.

A modification of the alkaline single cell gel electrophoresis (SCG) (Comet) assay was used to test the *in vivo* genotoxicity of biphenyl in mouse stomach, liver, kidney, bladder, lung, brain, and bone marrow. CD-1 male mice were sacrificed 3, 8, and 24 h after oral administration of 2000 mg/kg biphenyl. DNA damage was induced in all the organs studied, and biphenyl-induced increased DNA migration peaked at 24 h. (Sasaki *et al.*, 1997) The possible genotoxic and teratogenic actions of biphenyl (diphenyl) was investigated in two microbial systems and a metazoan model were used: diploid D7 strain of Saccharomyces cerevisiae; Salmonella typhimurium strains TA100, TA98, TA1535, TA1537, TA1538, TA1532, TA2636; and sea urchins (Paracentrotus lividus and Sphearechinus granularis). Severe toxicity resulted in all of the test organisms at levels greater than or equal to 10(-5) M (approximately 2 ppm). Biphenyl caused genetic effects in yeast with and without activating system. Biphenyl action on sea urchins resulted in developmental defects and mitotic abnormalities, following exposure of embryos or by pretreatment of sperm or eggs. The

minimal active concentration was 10-4 M (Pagano *et al.*, 1983). Biphenyl was not teratogenic in the rat (Khera et al, 1979).

1,1-Biphenyl was not mutagenic in reverse mutation tests in Salmonella and Eshcerichia coli and in a DNA repair test in E. coli (Anderson and Styles, 1978; Cline and McMahon, 1977; Hirayama et al., 1981). 1,1-Biphenyl did not induce chromosomal aberrations in Chinese hamster cells or unscheduled DNA synthesis in rat hepatocytes (Abe and Sasaki, 1977; Ishidate and Odashima, 1977; Brouns et al., 1979). 1,1-Biphenyl did induce forward mutation in mouse lymphoma cells and sister chromatid exchanges in Chinese hamster cells, although a dose-response relationship was not observed in the latter test (Wangenheim and Bolcsfoldi, 1988; Abe and Sasaki, 1977).

Naphthalene

The health hazards of naphthalene have been extensively studied. Recent reviews (European Union 2001, ATSDR 1996) indicate the most sensitive health effect endpoints are hemolytic anemia (humans) and respiratory effects including cancer (rodents). The naphthalene data are extensively discussed in the European Union (EU) Risk Assessment Document for Naphthalene (European Union 2001) and are summarized below.

An oral LD50 value for naphthalene in rats of approximately 1.8 g/kg has been reported. An oral dose of 1.0 g/kg for 2 days produced only slight eye effects in albino rats, whereas rabbits given 2000 mg/kg/day orally for 5 days developed cataracts; even after one or two oral doses of naphthalene at 1000 mg/kg, cataract formation had begun. Rabbits fed repeated doses of 1 g naphthalene/kg/day for up to 20 days showed browning of the lenses and eye humors, degeneration of the retina, and cataract formation.

Mice were exposed to an atmosphere containing 30 ppm naphthalene 6 hours/day for 6 months. This exposure did not elicit a significant increase in lung adenomas; however, an increased incidence of multiple pulmonary alveolar adenomas was observed upon histopathological examination. (Adkins *et al.*, 1986)

In a 2-year inhalation study, groups of male and female mice were exposed at 0, 10, or 30 ppm naphthalene, 6 hours/day, 5 days/week for 103 weeks.(National Toxicology Program 1992) In male mice, there was no increase in the incidence of tumors related to naphthalene exposure. In the female mice, the incidence of pulmonary alveolar/bronchiolar adenomas in the 30 ppm group (28/134, 21%) was significantly increased compared with controls (5/68, 7%). In the nose of both sexes, naphthalene was associated with an increase in the incidence and severity of minimal to mild chronic inflammation, metaplasia of the olfactory epithelium, and hyperplasia of respiratory epithelium. In the lung of both sexes, naphthalene exposure was associated with chronic inflammation. The U.S. National Toxicology Program (NTP) concluded that there was no evidence for the carcinogenicity of naphthalene in male mice and that there was some evidence of carcinogenic activity in female mice (National Toxicology Program 1992).

Groups of male and female rats were exposed by inhalation to 0, 10, 30, or 60 ppm naphthalene, 6 hours/day, 5 days/week for 105weeks. (National Toxicology Program 2000) The incidences of neuroblastoma of the olfactory epithelium, a rare neoplasm, occurred with positive trends in males and females and was considered to be related to naphthalene exposure. In males, the incidence of adenoma of the respiratory epithelium of the nose, another rare neoplasm, occurred with a positive trend and was significantly increased in all exposed groups; none occurred in chamber controls. In females, this neoplasm occurred in the 30 and 60 ppm groups. Because these neoplasms did not occur in the chamber controls they were considered to be related to naphthalene exposure. Increased incidences of nonneoplastic lesions of the nose included atypical hyperplasia, atrophy, chronic inflammation and hyaline degeneration of the olfactory epithelium; hyperplasia, squamous metaplasia, hyaline degeneration, and goblet cell hyperplasia of the respiratory epithelium; and glandular hyperplasia and squamous metaplasia. The NTP concluded that there was clear evidence of carcinogenic activity in male and female rats based on increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose (National Toxicology Program 2000).

No studies investigating effects on fertility are available. However, in a carcinogenicity study mice showed no histopathological changes in the epididymis, prostate, seminal vesicle, testis or ovary following inhalation of 30 ppm naphthalene vapor for 6 hours/day, 5 days/week for 104 weeks (estimated to be approximately 45 mg/kg/day) (National Toxicology Program 1992). Similarly, when male mice were given 133 mg/kg/day or 267 mg/kg/day for 90 days, no adverse effects on the testes could be discerned. (Shopp *et al.*, 1984)

In a well-reported developmental toxicity study groups of 25 rabbits were treated by gavage with 0, 20, 80 or 120 mg/kg/day of naphthalene on days 6-19 of gestation (Navarro *et al.*, 1992). Caesarean sections were conducted on day 30. There were no signs of maternal toxicity in any of the treatment groups and there were no differences in the number of resorptions, live and dead fetuses, litter size, fetal body weight and no increase in the incidence of external, skeletal or visceral malformations. The potential to produce developmental effects at maternally toxic doses was not assessed. The dose levels used were based on a preliminary study (which was cited in the above report) in which rabbits were treated with 0, 75, 150, 300 or 500 mg/kg/day, presumably according to the above protocol. Maternal deaths (at least 40%) occurred with 150 mg/kg/day and above. There were no signs of fetotoxicity. Pups were apparently not assessed for malformations. It is difficult to draw firm conclusions from the brief report of this preliminary study that did not assess occurrence of malformations.

In a well conducted unpublished study, groups of 18 rabbits were administered 0, 40, 200 or 400 mg/kg/day naphthalene by gavage on days 6-18 of gestation (Pharmakon, 1985). Caesarean sections were conducted on day 29. Two high dose animals aborted on days 18 and 23 of gestation, which was considered to be due to maternal toxicity. There were no naphthalene induced maternal deaths or statistically significant changes in maternal body weight. However, at 200 and 400 mg/kg/day increased dyspnoea, cyanosis and salivation were reported. Examination of the dams and offspring indicated no differences in the

number of implantations, post-implantation loss, number of live and dead fetuses, litter size, fetal body weight, or fetal sex distribution in any of the treatment groups. Overall, no developmental effects were observed at a naphthalene dose of up to 400 mg/kg/day, at which maternal toxicity was evident.

In a well conducted but poorly reported study groups of 25 female Sprague-Dawley rats were treated by gavage with 0, 50, 150 or 450 mg/kg/day naphthalene on days 6-15 of gestation (Navarro *et al*., 1991). Caesarean sections were performed on day 20. Maternal body weight gains (corrected for gravid uterine weight) were decreased by 22 and 29% with 150 and 450 mg/kg/day respectively. These animals were also lethargic and showed slow respiration rates during the treatment period. There were no differences in the number of corpora lutea per dam or the number of dead or live fetuses per litter in any treatment group. However there was a 2-fold increase in the number of resorptions per litter with the top dose compared to the controls. It was not stated whether these were considered to be early or late resorptions. Pups from top dose animals showed a slight increase in the number of litters with visceral malformations and slight dose-related increases in percentage of fetuses per litter with visceral malformations was also noted. However these increases, which were principally due to increased incidence of enlarged lateral ventricles in the brain, were not statistically significant. Overall this study provides some evidence of fetotoxicity occurring at maternally toxic doses with no fetotoxicity occurring at doses which were not maternally toxic. The dose levels used were based on a preliminary study (cited in the above report) in which rats were treated with 0, 100, 400, 500, 600 or 800 mg/kg/day, presumably according to the protocol above. Severe maternal toxicity was noted with the top two doses. With the top dose 67% of dams died and total resorptions occurred in 33% of the survivors. No further details were presented of the toxicity seen with 600 mg/kg/day. "Slight" maternal and fetal toxicity was noted with 400 and 500 mg/kg/day although no details were given. Pups were not assessed for malformations. Overall fetal toxicity was apparently observed at maternally toxic doses. However it is difficult to draw firm conclusions from the brief report of this preliminary study which did not assess the occurrence of malformations.

In a poorly conducted study in CD-1 mice, groups of 50 females were treated by gavage with 0 or 300 mg/kg/day naphthalene on days 7-14 of gestation (Plasterer *et al*, 1985). Dams showed a 15% decrease in survival and a 26% reduction in body weight. There was a statistically significant decrease (18%) in the number of live pups/litter, although a corresponding increase in the number of dead pups/litter was not noted. There was no change in pup weight. Gross examinations were apparently performed on the pups but it is not clear if visceral and skeletal examinations were conducted. The number of resorptions was not assessed. Overall, evidence of fetal toxicity was observed in this limited study at a dose producing severe maternal toxicity.

Naphthalene was not mutagenic in Salmonella typhimurium (strains TA98, TA100, TA1535, TA1537, (Kraemer *et al.*, 1947; McCann *et al.*, 1975) UTH 8414, or UTH 8413 (18)) either in the presence or absence of a hamster or rat liver (S9) metabolizing system. No evidence for naphthalene mutagenesis was obtained in studies with cultured embryonic rodent (Rhim, 1974; Freeman, 1973) or mammary gland (Tonelli, 1979) cells.

Dicyclopentadiene

DCPD is a mid-range (C10) dicyclic alkene found at varying levels in many of the category streams. DCPD is an OECD SIDS chemical with an established screening data set and hazard profile for human health and environmental effects (OECD, 1998). The toxicology of DCPD has been reviewed by Cavender (1994a) and ECETOC (1991) and a summary of this information follows. Details of key studies pertinent to the OECD SIDS health effects endpoints are provided in the robust summaries that accompany the Olefins Panel Resin Oils and Cyclodiene Dimer Concentrates test plan. The available health effects information indicates that DCPD is moderately toxic by relevant routes of exposure. Acute lethal oral doses in animal species are variable ranging from 0.19 g/kg in the mouse to approximately 1.2 g/kg in cattle. Lethal vapor concentrations are also variable, ranging, for 4-hour exposures, from 145 ppm for the mouse, to approximately 770 ppm for the guinea pig and rabbit. Substantially saturated vapor concentrations (2500 ppm) are lethal to rats in 60 minutes (Gage, 1970). However, the 4-hour rat LC_{50} is about 660 ppm; three of four rats survived ten 6-hour daily exposures at 250 ppm, and all survived 15 such exposures at 100 ppm. (Gage, 1970) Dogs, guinea pigs, and rabbits were more resistant than mice. (Kinkead, *et al.*, 1971) All species followed a general pattern of eye irritation, loss of a considerable degree of coordination, and if death ensued, convulsions. (Kinkead, *et al.*, 1971; ECETOC, 1991; Gage, 1970). Similar to other hydrocarbons, the predominant acute systemic effect is on the central nervous system; DCPD produces initial stimulation followed by prolonged depression. DCPD has a disagreeable odor similar to camphor and has reportedly resulted in headaches in workers following prolonged exposure to low vapor concentrations. DCPD is also irritating when directly applied to the skin and eyes and may be an aspiration hazard.

Several studies have evaluated DCPD for repeated exposure effects. The most consistent effect at non-lethal doses was to the kidneys of male rats but some studies also found effects to the lung, liver, gastrointestinal tract, and adrenal gland. In feeding studies, DCPD given for up to 90 days to mice and rats did not result in treatment-related effects at nominal dietary concentrations up to 273 ppm or 750 ppm, respectively. Dogs in a similar study exhibited some evidence of gastro-intestinal disturbance at the highest dietary concentration (1,000 ppm nominal). In the most recent study conducted by gavage and according to OECD Guideline 422 , daily exposure to 4, 20, or 100 mg/kg DCPD produced a variety of effects to male and female rats (JETOC, 1998). Two females (of ten) in that received 100 mg/kg died during treatment and (all) males and surviving females exhibited slight suppression of body weight gain and decreased feed consumption. Male rats of the high dose group demonstrated increase in liver enzymes, increased liver and kidney weight, and microscopic findings of single cell necrosis in the liver and hyaline droplets and renal tubular changes in the kidney. The kidney microscopic changes were also observed in the male rats that received 4 and 20 mg/kg DCPD. Both males and females in the 100 mg/kg group and males in the 20 mg/kg group also exhibited increase in fatty droplets in the adrenal glands. The no observed effect level doses for repeat dose toxicity for this study were considered to be 20 mg/kg/day for females and less than 4 mg/kg/day for males.

Repeated inhalation exposure of laboratory animals to DCPD vapor also produced kidney lesions in male rats of several studies. The kidney lesions described in these studies give the

appearance of the male rat specific disease hyaline droplet nephropathy, a condition not considered relevant to humans. Lung lesions described as chronic pneumonia and bronchiectasis was reported in rats exposed to 35 and 74 ppm (Kinkead *et al*., 1971); however in a second study (Bevan *et al*., 1992), no lung lesions were observed in rats repeatedly exposed to 50 ppm DCPD.

DCPD is not selectively toxic to rodent reproduction or the developing embryo/fetus. In a reproductive/developmental toxicity screening study conducted by oral gavage (JETOC, 1998), no effects were noted on reproductive parameters at up to 100 mg/kg. This dose, however, was lethal to 2 (of 10) female rats and 2 rats of this group (presumably the same animals) lost 100% of their litters during lactation (days 1-4). A low viability index and tendency to lower birth weight and body weight gain were observed in neonates in the highest dose group. The no observed effect level doses for this study were 100 mg/kg/day for parental males and 20 mg/kg/day for parental females and offspring. The NTP evaluated the potential reproductive toxicity of orally (gavage) administered DCPD (10, 30, or 100 mg/kg) in rats using a continuous breeding protocol (Jamieson *et al*., 1995). DCPD at 100 mg/kg produced lower pup weights, increased pup mortality, fewer pups born alive, and increased cumulative days to litter. In the 30 mg/kg group, only a slight (4%) reduction in the average female pup weight was observed. There were no reproductive effects observed in the 10 mg/kg group. Epididymal sperm density, percent motility, percent abnormal sperm, spermatids per milligram of testis, and total spermatids per testis were not affected by the administration of DCPD at dose levels employed in this study. There was decreased F2 pup weight in the 100 mg/kg group of the second generation. At the doses that yielded reproductive effects, parental animals exhibited effects on liver and kidney; hence the DCPD reproductive effects that were observed in this study were not considered by NTP to be selective. A 3-generation reproduction study of DCPD administered to rats in the diet at 80 and 750 ppm resulted in no deleterious effects on reproductive processes or general condition of the rats and no evidence of dose-related teratologic effect over three successive generations with two matings per generation (Hart, 1980).

Developmental toxicity range-finding studies were conducted by NTP in New Zealand White rabbits and Sprague-Dawley rats (Gulati *et al*., 1993a,b). DCPD administered by gavage at 25, 100, 200, 300, or 400 mg/kg to rabbits caused maternal toxicity at 200 mg/kg and higher doses. Gross deformities were evident at 400 mg/kg but no other developmental endpoints were significantly affected. Rats were administered DCPD at 50, 200, 300, 400, and 500 mg/kg by gavage. Body weights were significantly decreased at two time points and for body weight gain throughout the treatment for rats in the 50 and 200 mg/kg groups. Clear maternal toxicity, including maternal death, was observed at 200 mg/kg and higher doses (3/7 in the 200 mg/kg group, 8/9 in the 300 mg/kg group, and all in the 400 and 500 mg/kg groups were found dead by gestation day 9). Developmental toxicity in the form of decreased fetal weight was observed in the 200 mg/kg group. In a rat teratology study there were no effects on pregnant dams from dietary administration of 80, 250, or 750 ppm DCPD and no compound-induced terata, variation in sex ratio, embryo toxicity or inhibition of fetal growth and development (Hart, 1980). DCPD is not toxic to genetic mechanisms either in bacterial or mammalian systems. Tests for mutations and chromosomal effects have been negative for DCPD. DCPD has not been evaluated for carcinogenic effects.

Other Components

The biological activity of DCPD is expected to be similar to that of other physicochemically similar C8 to C12 cycloalkenes. There is less information available, however, for other mono- and dicyclic alkenes and their substituted derivatives as these substances are of lesser commercial interest. The toxicology properties of cycloalkenes is reviewed by Cavender (1994a). The available information for C8 to C12 cycloalkenes indicate these hydrocarbons show similar acute toxicity profiles as DCPD in terms of lethal dosages and clinical signs dominated by CNS effects. The liquid cycloalkenes in this range are also considered aspiration hazards. These hydrocarbons exhibit irritation effects with some producing severe and corrosive effects to the skin (e.g. cyclooctadiene). Some members are also skin sensitizers. There is very limited reliable information available on the toxic effects of C8 to C12 cycloalkenes following repeated exposure. A few studies have been conducted on limonene (a C10 cycloalkene that occurs in the oil of many plants). Decreases in body weight and non-specific systemic effects were noted in mice and dogs that received oral doses of limonene for up to 1 to 6 months. In male rats, limonene resulted in formation of hyaline droplets in the kidneys, a similar finding with DCPD.

The C8 to C12 aromatic hydrocarbons in general show qualitatively similar toxicological properties as the C8 to C12 cycloalkenes (Cavender, 1994a,b). There are quantitative differences, however, between these hydrocarbons with the cycloalkenes producing greater toxicity at comparable dosages. The available information for solvents that are mixtures of C8 to C12 aromatic hydrocarbons indicate in general that this range of aromatic hydrocarbons are: of low to moderate acute toxicity producing transient CNS effects at high doses, of low repeated exposure systemic toxicity, not genotoxic, and not selectively toxic to the developing fetus, embryo, or reproductive system. The specific assessment of the available toxicology information for the C8 to C12 aromatic hydrocarbons is to be included in the International Hydrocarbon Solvents Consortium C9 Aromatic Hydrocarbon Solvents and C10+ Aromatic Hydrocarbon Solvents categories and will not be discussed more specifically in this test plan.

In addition to the existing information on DCPD as the dominant and / or representative cycloalkene and on C8 to C12 aromatic hydrocarbons, there is also some limited information available on streams that consist of both kinds of hydrocarbons. As expected, the toxicological properties of the streams are not dissimilar to that of this range of cycloalkenes and aromatic hydrocarbons. Resin-Former Feedstock, a test sample that consisted of 50-60% DCPD, 15-20% cyclopentadiene/methyl cyclopentadiene dimer, < 2% butadiene dimer, 10-12% styrene, < 2% xylene, and < 2% cyclopentadiene, exhibited low acute toxicity with CNS effects presented (Rausina, 1983; Gordon, 1983a). In addition, it possesses low to moderate toxicity following repeated exposure with evidence of CNS (likely acute), liver and kidney (hydrocarbon nephropathy) effects, and generally an absence of genotoxic effects including an *in vivo* mouse micronucleus test (Rausina, 1984; Gordon, 1983b; Papciak and Goode, 1984; Brecher and Goode, 1984; Khan and Goode, 1984). This material did exhibit positive activity in one *in vitro* system, a test of cell transformation in mouse embryo cells (Brecher and Goode, 1983). Details on these studies are provided in The Resin Oils and Cyclodiene Dimer Concentrates category robust summaries.

To supplement the above data, the Olefins Panel Resin Oils and Cyclodiene Dimer Concentrates test plan proposes testing two representative streams, Low DCPD Resin Oil and DCPD/Codimer Concentrate. This testing will provide SIDS datasets for these streams which are relevant to the Fuel Oils category.

The details of the strategy are as follows:

Existing data on Fuel Oil streams and stream components, as well as new data resulting from other testing programs on components present in significant amounts in the streams of the Fuel Oils Category will be evaluated.

Stream Components will be evaluated from data from the sources indicated below:

- Dicyclopentadiene: DCPD is sponsored in the OECD SIDS program by Japan. A SIAR has been submitted.
- Naphthalene: A risk assessment is being conducted under the EU Existing Substances Directive and is expected to be completed soon.
- Biphenyl: This material has been volunteered under the HPV Challenge program by the SOCMA Biphenyl Working Group for 2003.
- Mixture of C5 olefins and aliphatic hydrocarbons: Included in the Olefins Panel C5 Non-Cyclics Category. A test plan was submitted to EPA on November 7, 2001.
- Heavy Fuel Oils (Petroleum): These chemically similar materials have been volunteered under the HPV Program by the American Petroleum Institute for 2003.
- C8 to C12 aromatic hydrocarbons: Included in the International Hydrocarbon Solvents Consortium C9 Aromatic Hydrocarbon Solvents and C10+ Aromatic Hydrocarbon Solvents categories to be addressed in OECD SIDS (ICCA). Test plans are being submitted to EPA in 2001 and 2002.
- C7 to C10 aliphatic hydrocarbons: Included in the International Hydrocarbon Solvents Consortium C7 to C9 Aliphatic Hydrocarbon Solvents and C9 to C13 Aliphatic Hydrocarbon Solvents categories to be addressed in OECD SIDS (ICCA). Test plans are being submitted to EPA in 2001 and 2002.
- The DCPD stream studies for the Olefins Panel Resin Oils and Cyclodiene Dimer Concentrates Category are described above. The test plan for this category will be submitted in 2001.

Fuel Oils Streams

Toxicology data for oral, dermal, and inhalation routes of exposure are available for the following mixed process streams that represent typical Fuel Oils. Robust summaries of relevant studies have been prepared for the following:

- Light Pyrolysis Fuel Oil

- Aromatic Pyrolysis Oil, Rerun Tower Bottoms
- Biphenyl Feedstock
- Coal Derived Fuel Oils

Existing studies of these Fuel Oils steams are indicated in Table 4, along with supporting data from studies on related materials, including two coal derived experimental fuel oils with similar composition. Mutagenicity studies, including *in vivo* mouse micronucleus assays for these streams, indicate the potential to produce point mutations, unscheduled DNA synthesis, and cell transformations, especially for heavy fuel oils. Repeated dose studies of up to lifetime duration (i.e., 28 months) indicate the potential for development of skin tumors (dermal exposure) and decreased body weight and hematological effects (dermal and inhalation exposures). Histopathological changes were observed in kidneys, adrenal glands, liver, lung, thymus and bone marrow. Results from the limited number of reproductive studies on these and related materials indicate little effect on reproductive capacity or performance; however, there were increases in specific malformations observed in developmental toxicity studies of related materials. The observed effects are likely attributable to the polycyclic aromatic hydrocarbon content that can range upward of 5%. Taken as a whole, these data suggest that the most sensitive health effects for Fuel Oils are due to the C10-12 components and polycyclic aromatic hydrocarbon content. The mammalian health hazards of these materials have been adequately characterized and no further testing will be conducted.

B. Environmental / Aquatic Effects and Test Plan Strategy

PHYSICOCHEMICAL PROPERTIES

The physicochemical (PC) endpoints for the HPV Chemical Program include:

- Melting Point
- Boiling Point
- Vapor Pressure
- Water Solubility
- Octanol/Water Partition Coefficient (K_{ow})

Although some of these data for products in the Fuel Oils Category exist, not all of these endpoints are defined, and a comprehensive and consensus database for chemicals that represent product streams in this category does not exist. Therefore, calculated PC data for selected chemical components in this category will be developed using a computer model to provide a consistent, representative data set. Also, existing measured data will be identified and provided where readily available. In addition, selected PC data will be developed for two products: one representative of a lighter product containing a larger proportion of lower molecular weight chemical, and the other representative of a heavier product.

Calculated PC data for selected chemical components in the Fuel Oils Category will be developed using the EPIWIN© computer model (EPIWIN, 1999), as discussed in the U.S. EPA document entitled *The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program* (US EPA, 1999a). The use of computer

modeling for the development of these data is justified since components of the streams in this category are chemically related and expected to exhibit relatively similar environmental properties. In addition, for all the chemicals selected to represent products in this category, a calculated dataset provides a common method for the development of these values.

Boiling point, melting point, and vapor pressure ranges will be determined using the MPBPVP subroutine in EPIWIN. K_{ow} and water solubility will be calculated using KOWIN and WSKOW subroutines, respectively. There is more information on calculating data for the HPV chemical program in the EPA document titled, *The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program*.

Because the HPV substances covered under the Fuel Oils Category testing plan are mixtures containing differing compositions, it is not possible to develop or calculate a single numerical value for each of the physicochemical properties. For example, a product that is a mixture of chemicals does not have a melting point, but rather a melting range. Calculated values for PC properties will be represented as a range of values according to the product's component composition.

Robust summaries characterizing the PC endpoints will be prepared upon completion of the proposed testing, and will include the calculated data and testing results.

ENVIRONMENTAL FATE

Environmental fate endpoints for the HPV Chemical Program include:

- Biodegradation
- Photodegradation
- Hydrolysis
- Fugacity

There are no data available on the biodegradability of products from this category. However, there are data for pure chemicals and complex products containing several chemicals not in this category, but which can be found in products from this category. These data can be used to initially assess the biodegradability of products in this category. The data suggest that fuel oil products have the potential to biodegrade to a significant extent. To confirm and characterize the potential of products in this category to biodegrade, two products will be tested.

Data and/or information in the form of a technical discussion will be provided for photodegradation. Chemicals in this category are not subject to hydrolysis at measurable rates, therefore information for this endpoint will be summarized in a technical review document.

Equilibrium models are used to calculate chemical fugacity, which can provide information on where a chemical is likely to partition in the environment. These data are useful in identifying environmental compartments that could potentially receive a released chemical. Fugacity data can only be calculated for individual chemicals. For the HPV Program,

environmental partitioning data will be calculated for selected chemical components of the products in this category.

A preliminary evaluation of chemicals in the Fuel Oils Category suggests that they will partition largely to the air and soil, and therefore their fate in these compartments is of environmental interest. Because the air phase may be a compartment that could potentially receive many of the chemical components in this category, data characterizing their potential for physical degradation in the atmosphere will be developed (this is discussed below under photodegradation).

1. Biodegradation

A biodegradation study was identified for a product in the Fuel Oils Category. Although there are only limited data, several studies were identified for component chemicals found in fuel oil products and for products not in this category. The product data are for complex substances that contain several hydrocarbons, some or all of which are found in this category. Results from these studies can be used as read across data to support an initial assessment of the persistence of fuel oil products in the environment. Although these data alone may not characterize the potential biodegradability of the chemically complex products in this category, they do provide an initial understanding of the fate of these products. The data suggest that products in this category have the potential to biodegrade to a significant extent.

A biphenyl feedstock (reported as CAS# 68989-41-3), a fuel oil product, exhibited 57% biodegradation after 28 days using an OECD (Organization for Economic Co-ordination and Development) 301 D, closed bottle test guideline. The chemical component biodegradation data are for benzene and two isomers of xylene, while the complex product data are for a primarily C8 alkene product, a primarily C9 alkylbenzene product, and a primarily naphthalene/alkylnaphthalene product. The pure chemicals exhibited 63 to 88% biodegradation after 28 days using an OECD 301 F, manometric respirometry test guideline. The data for the chemically complex products ranged from 29 to 78% for the same duration, using the same test procedure.

The manometric respirometry test (OECD guideline 301F) uses a continuously-stirred, closed test system, which is recommended when assessing the biodegradability of volatile materials like those in this category. This test is also recommended when evaluating complex products containing several chemical species, some of which may be minimally water-soluble.

To address the potential biodegradability of products in this category, the Panel proposed to test two products via manometric respirometry (OECD Guideline 301F). One product will be representative of a lighter product containing a larger proportion of lower molecular weight components and the second representative of a heavier product containing a larger proportion of higher molecular weight components. The data from the proposed testing will be compared to the data discussed above to determine whether products in this category are as readily biodegraded as suggested by those data.

2. Photodegradation – Photolysis

Direct photochemical degradation occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then the resultant excited state of the chemical may lead to its transformation. Simple chemical structures can be examined to determine whether a chemical has the potential for direct photolysis in water. First order reaction rates can be calculated for some chemicals that have a potential for direct photolysis using the procedures of Zepp and Cline (1977).

To develop information or data that will characterize the potential of products in this category to undergo direct photochemical degradation, the existing product chemical composition data and composition data that will be developed for the two products identified for biodegradation testing will be evaluated together to select a subset of chemicals that adequately represents products in this category. The selection process will consider chemical carbon number range, hydrocarbon type, and chemical structure. The UV light absorption of the selected chemicals will then be evaluated to identify those chemicals with a potential to degrade in solution. When possible, first order reaction rates will be calculated for those chemicals identified to have a potential for direct photolysis in water. The results of the calculations will be summarized in a technical discussion for this endpoint. If instead, a low potential for direct photolysis is suggested by the evaluation, a technical discussion will be prepared to summarize the findings.

3. Photodegradation – Atmospheric Oxidation

Photodegradation can be measured, EPA identifies OECD test guideline 113 as a test method (EPA, 1999b)' or estimated using models accepted by the EPA (EPA, 1999a). An estimation method accepted by the EPA includes the calculation of atmospheric oxidation potential (AOP). Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect photodegradation. AOPs can be calculated using a computer model. Hydrocarbons, such as those in the Fuel Oils Category, have the potential to volatilize to air where they can react with hydroxyl radicals (OH-).

The computer program AOPWIN (atmospheric oxidation program for Microsoft Windows) (EPIWIN, 1999) is used by OPPTS (Office of Pollution Prevention and Toxic Substances). This program calculates a chemical half-life based on an overall OH- reaction rate constant, a 12-hr day, and a given OH- concentration. This calculation will be performed for representative chemical components of products in the Fuel Oils Category. The existing product chemical composition data and composition data that will be developed for the two products identified for biodegradation testing will be evaluated together to select a subset of chemicals that adequately represents products in this category. The selection process will consider chemical carbon number range, hydrocarbon type, and chemical structure. The resulting calculations will be summarized in a robust summary for this endpoint.

4. Hydrolysis

Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Neely, 1985).

Chemical stability in water can be measured (EPA identifies OECD test guideline 111 as a test method) or estimated using models accepted by the EPA (EPA, 1999a). An estimation method accepted by the EPA includes a model that can calculate hydrolysis rate constants for esters, carbamates, epoxides, halomethanes, and selected alkylhalides. The computer program HYDROWIN (aqueous hydrolysis rate program for Microsoft windows) (EPIWIN, 1999) is used for this purpose by OPPTS. However, all of the chemical structures included in the Fuel Oils Category are hydrocarbons. That is, they consist entirely of carbon and hydrogen. As such they are not expected to hydrolyze at a measurable rate.

A technical document will be prepared that discusses the potential hydrolysis rates of chemicals in this category, the nature of the chemical bonds present, and the potential reactivity of this class of chemicals with water.

5. Fugacity Modeling

Fugacity based multimedia modeling can provide basic information on the relative distribution of chemicals between selected environmental compartments (i.e., air, soil, sediment, suspended sediment, water, biota). The U.S. EPA has acknowledged that computer modeling techniques are an appropriate approach to estimating chemical partitioning (fugacity is a calculated endpoint and is not measured). A widely used fugacity model is the EQC (Equilibrium Criterion) model (MacKay *et al.*, 1996). The U.S. EPA cites the use of this model in its document titled *Determining the Adequacy of Existing Data* (EPA, 1999b), which was prepared as guidance for the HPV Program.

In its document, the U.S. EPA states that it accepts Level I fugacity data as an estimate of chemical distribution values. The input data required to run a Level I model include basic physicochemical parameters; distribution is calculated as percent of chemical partitioned to 6 compartments described above within a defined unit world. Level I data are basic partitioning data that allow for comparisons between chemicals and indicate the compartment(s) to which a chemical is likely to partition.

The EQC Level I is a steady state, equilibrium model that utilizes the input of basic chemical properties including molecular weight, melting point, vapor pressure, and water solubility to calculate distribution within a unit world. This model will be used to calculate distribution values for representative chemical components in products from this category. Existing product chemical composition data and composition data that will be developed for the two products identified for biodegradation testing will be evaluated together to select a subset of chemicals that adequately represents products in this category. The selection process will consider chemical carbon number range, hydrocarbon type, and chemical structure. A

computer model, EPIWIN version 3.04 (EPIWIN, 1999), will be used to calculate the physicochemical properties needed to run the Level I EQC model. The resulting calculations will be summarized in a robust summary for this endpoint.

AQUATIC TOXICITY

Aquatic toxicity endpoints for the HPV Chemical Program include:

- Acute Toxicity to a Freshwater Fish
- Acute Toxicity to a Freshwater Invertebrate
- Toxicity to a Freshwater Alga

An acute invertebrate toxicity study is available on a product from this category. There are no fish or alga toxicity data available for products in this category. However, there are read across data to initially characterize these two endpoints for chemicals found in products from this category and complex products that contain chemicals found in products from this category. The data suggest that products in this category have the potential to exhibit a moderate range of toxicity for the three aquatic toxicity endpoints. The use of data from selected read across materials to products in this category can be justified for the following reasons:

- Individual chemicals and complex products used for read across purposes contain a chemical class (i.e., aromatics) that is found in products from this category.
- Individual chemicals and complex products used for read across purposes have a carbon number or carbon number range that falls within the range of carbon numbers found in products from this category.
- Individual chemicals and complex products used for read across purposes as well as a product in this category are composed of chemicals that all act by a similar mode of toxic action.

The aquatic toxicity of products in the Fuel Oil Category are expected to fall within a narrow range regardless of the varying carbon number range and constituent composition of those products. This is expected because the constituent chemicals of those products are neutral organic hydrocarbons whose toxic mode of action is non-polar narcosis. The mechanism of short-term toxicity for these chemicals is disruption of biological membrane function (Van Wezel and Opperhuizen, 1995), and the differences between toxicities (i.e., LC/LL_{50}, EC/EL_{50}) can be explained by the differences between the target tissue-partitioning behavior of the individual chemicals (Verbruggen *et al*., 2000).

The existing fish toxicity database for hydrophobic neutral chemicals supports a critical body residue (CBR, the internal concentration that causes mortality) of between approximately 2-8 mmol/kg fish (wet weight) (McCarty and MacKay, 1993; McCarty *et al*., 1991). When normalized to lipid content the CBR is approximately 50 umol/g of lipid for most organisms (Di Toro *et al*., 2000). Because most of the products in this category are composed of complex combinations of relatively similar series of homologous chemicals, their short-term toxicities are expected to fall within the range of toxicity demonstrated by the chemicals and products summarized in this test plan. Therefore, these existing data that are believed to form

a sufficiently robust dataset to initially characterize the expected range of aquatic toxicity for products in this category.

An acute invertebrate study is available for a biphenyl feedstock product (reported as CAS# 68989-41-3), a fuel oil product, in this category. The 48-hour LL50 value for *Daphnia magna* is reported as 23.6 mg/L. Because the product tested was a complex hydrocarbon material containing several chemical components, exposure solutions for each treatment level were developed as water accommodated fractions and the results reported as lethal loading (LL) values, which is the procedure recommended by OECD for these types of materials (OECD, 1999).

Table 6 lists the chemical and product data that do not belong to this category, but are being used to support the initial characterization of the aquatic toxicity of this category. The acute toxicity data are for fish and an invertebrate, and fall within an effect range of 1.1 to 23.6 mg/L.

To address whether the expected toxicity for the three aquatic endpoints falls within the range of data presented above for with this category, the Panel proposes to test two products. One product will be representative of a lighter product containing a larger proportion of lower molecular weight components and the second will be representative of a heavier product containing a larger proportion of higher molecular weight components. Acute fish, acute invertebrate, and alga toxicity tests will be conducted for each product. In addition, the chemical composition of the products tested will be characterized to the degree that predominant chemical constituents and/or carbon number and chemical class (i.e., olefin, paraffin, aromatic) will be identified.

IV. TEST PLAN SUMMARY

The existing Fuel Oils stream data, existing components data, and data under development by the American Chemistry Council for other categories under the HPV program, by other HPV consortia, and by the OECD SIDS program, will be sufficient to characterize the human health endpoints of the range of products in this category in satisfaction of HPV program requirements. Environmental fate and effects endpoints will be thoroughly characterized and a comprehensive and consensus physicochemical database for products in this category will be developed.

Aquatic testing, modeling, technical discussions, and physicochemical data will be developed for the Fuel Oils Category as described below, and as detailed in Table 5.

- Conduct acute aquatic toxicity studies on two streams representative of the light and heavy ends of the fuel oils product spectrum.
- Conduct biodegradation studies on a product from each of the same two streams.
- Prepare a technical discussion on the potential of chemical components comprising streams in this category to photodegrade.

- Prepare a technical discussion on the potential of chemical components comprising streams in this category to hydrolyze.
- Calculate fugacity data for selected chemical components of streams in this category.
- Calculate physiochemical data as described in the EPA document titled *The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program* and identify readily available data. Develop relevant measured physicochemical data for the two products selected for environmental fate and effects testing cited above.

Summaries of the results will be developed once the data and analyses are available. This test plan is expected to provide data sufficient to characterize the human health effects and environmental fate and effects endpoints for the Fuel Oils Category under the program.

V. OTHER SUPPORTING DATA

Additional data for components of the Fuel Oils streams that will provide support for this category will be collected by other test plans within the HPV program (see Table 7), by other consortia participating in the HPV or ICCA programs, or from chemicals sponsored in the OECD SIDS program. These include:

- Dicyclopentadiene: DCPD is sponsored in the OECD SIDS program by Japan. A SIAR has been submitted.
- Naphthalene: A risk assessment is being conducted under the EU Existing Substances Directive and is expected to be completed soon.
- Biphenyl: This material has been volunteered under the HPV Challenge program by the SOCMA Biphenyl Working Group for 2003.
- Heavy Fuel Oils (Petroleum): These chemically similar materials have been volunteered under the HPV Program by the American Petroleum Institute for 2003.
- C8 to C12 aromatic hydrocarbons: Included in the International Hydrocarbon Solvents Consortium C9 Aromatic Hydrocarbon Solvents and C10+ Aromatic Hydrocarbon Solvents categories to be addressed in OECD SIDS (ICCA). Test plans are being submitted to EPA in 2001 and 2002.
- C7 to C10 aliphatic hydrocarbons: Included in the International Hydrocarbon Solvents Consortium C7 to C9 Aliphatic Hydrocarbon Solvents and C9 to C13 Aliphatic Hydrocarbon Solvents categories to be addressed in OECD SIDS (ICCA). Test plans are being submitted to EPA in 2001 and 2002.
- The DCPD stream studies for the Olefins Panel Resin Oils and Cyclodiene Dimer Concentrates Category are described above. The test plan for this category will be submitted in 2001.

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Table 1. CAS Numbers used in the Fuel Oils Category

CAS #	CAS Inventory Name
64741-62-4	Clarified oils, petroleum, catalytic cracked
64742-90-1	Residues, petroleum, steam-cracked
68131-05-5	Hydrocarbon oils, process blends
68409-73-4	Aromatic hydrocarbons, biphenyl-rich
68475-80-9	Distillates, petroleum, light steam-cracked naphtha
68513-69-9	Residues, petroleum, steam-cracked light
68514-34-1	Hydrocarbons, C9-14, ethylene-manuf.-by-product
68527-18-4	Gas oils, petroleum, steam-cracked
68921-67-5	Hydrocarbons, ethylene-manuf.-by-product distn. residues
69013-21-4	Fuel oil, pyrolysis
69430-33-7	Hydrocarbons, C6-30
8002-05-9	Petroleum

Table 2. Steams Included in the Fuel Oils Category

Olefins Industry Stream
Heavy Pyrolysis Fuel Oil from the Ethylene Process Unit
Light Pyrolysis Fuel Oil from the Ethylene Process Unit
Quench Oil from the Ethylene Process Unit water quench system
Pyrolysis Fuel Oil from Pyrolysis Gasoline Distillation
Combined Fuel Oil from the Ethylene & Pyrolysis Gasoline
Combined Fuel Oil from Benzene HDA and Pyrolysis Fuel Oils
Hydrotreated Flux Oil
Biphenyl Concentrate

Table 3. Typical Stream Compositions for Fuel Oils Category

Component (See note 1, 2 and 3 at the bottom of this table.)	Heavy Pyrolysis Fuel Oil from the Ethylene Process Unit*	Quench Oil from the Ethylene Process Unit water quench system	Pyrolysis Fuel Oil from Pyrolysis Gasoline	Combined Fuel Oil from Ethylene & Pyrolysis Gasoline	Light Pyrolysis Fuel Oil from the Ethylene Process Unit	Hydro-treated Flux Oill	Biphenyl Concen-trate	Combined Fuel Oil from Benzene HDA & Pyrolysis Fuel Oils
Composition, wt %								
1,3-Butadiene		0.1 - 0.3						
C6 Non-aromatics				0.2 - 3.1				
C5s and Lighter				1.8				
C6 and Lighter								0.2
Benzene		0.1		0.2 – 4				0. 1 - 0.3
C7 Paraffins & Napththenes				3				
Toluene		5		0.2 – 1.3			1 - 8	
C8 Paraffins & Naphthenes				6.1				
Ethylbenzene		5					1	
C8 Aromatics				0.4 – 2.6				
Xylenes, Mixed		5					2	
Styrene		0 - 5		0.9				
C9 Aromatics			2	12.6				
Other Benzenes to Naphthalene				14.5				11
C9 Paraffins & Naphthenes				12.6				
C10+ (NOS)							20 – 25	
Trimethylbenzenes				1				
Dicyclopentadiene			20	0.9				7.5 - 11.7
C10 & C11 Codimers of C5&C6			30					
Indane (Indan)				1.5				
2,3-Benzindene				2 – 5				5 - 6.4
Methyl Dicyclopentadiene				0.9				
C10 Aromatics				32.1		7.6		
C11 Isoparaffins						26.7		
1,4-Dimethyl-2-ethylbenzene						1.9		
1,4-Methyl-t-butylbenzene						2.8		
Undecene-1						0.8		
Indene		5	2	0.7 - 0.8	5 – 15			3.8
1,2,3,5-Tetramethylbenzene (Isodurene)						2.4		
Methyl Indenes				5.6				0.2 – 2
C12 Isoparaffins						5.9		
4-Methylindan						2.3		
2-Methylindan						4.4		
1,3-Methyl-n-butylbenzene						5.4		
1,3-Di-i-propylbenzene						7.8		
1,3-Diethyl-5-methylbenene				1.5				
Dimethyindan				4.0				
Dimethylindene				5.4				

Component (See note 1, 2 and 3 at the bottom of this table.)	Heavy Pyrolysis Fuel Oil from the Ethylene Process Unit*	Quench Oil from the Ethylene Process Unit water quench system	Pyrolysis Fuel Oil from Pyrolysis Gasoline	Combined Fuel Oil from Ethylene & Pyrolysis Gasoline	Light Pyrolysis Fuel Oil from the Ethylene Process Unit	Hydro-treated Flux Oill	Biphenyl Concen-trate	Combined Fuel Oil from Benzene HDA & Pyrolysis Fuel Oils
Composition, wt %								
n-C13				1.3				
Methylcyclopentadiene Dimers				5.1				
C11 Aromatics						4.6		
Naphthalene	0 - 4	0.7 - 10	7	10 - 47	30 - 60	7.3	1 - 4	7 - 13.2
1,3,5-Triethylbenzene						2.4		
C12 Aromatics						3.5		
Dodecene-1						0.7		
C13 Isoparaffins						0.6		
C7-C18 Cyclic Olefins		65.0						
Methylnaphthalene				3.8 - 30			1	
2-Methylnaphthalene			2					0.1 – 13
1-Methylnaphthalene			2					9
Fluoranthene		0 - 1.1						
1,1'-Biphenyl		0.5 - 5	6	1.1 - 5.1			65 - 95	25 - 34.6
Ethyl Naphthalene's				0.8				1.5 - 4
Substituted Napthalenes			13					
1-Ethylnaphthalene			8					
Dimethylnapthalenes			8	3.8				
Acenaphthylene		0.1 - 6.9						
Diphenylethane				2 – 7				
Acenaphthene		0.1 - 1.3						2
Fluorene				3				
C10 Parafins & Naphthenes				1.1				
C14+						2.5		
Phenanthrene				5				7
Anthracene		10		1 - 5				2
Heavy Hydrocarbons and Polycyclic Aromatics			7.0					
Terphenyls								2.5
Methylbiphenyls				5 - 10			1 - 3	6.2
>C18 Cyclic Olefins		5						
1,2-Dihydroacenaphthylene			1					

NOS not otherwise specified
* Consists of C10+ and polycyclic aromatic hydrocarbons, NOS.
Note 1: The composition data shown are composites of reported values.
Note 2: The balance of these streams is expected to be other hydrocarbons that have boiling points in the range of the listed components.
Note 3: The listed highs and lows should not be considered absolute values for these limits. They are instead the highs and lows of the reported values.

Table 4. Existing Data To Use for Evaluation of Streams in Fuel Oils Category

Robust summaries for available data submitted separately.

	Human Health Effects						Environmental Toxicity			Physical Chem.	Environmental Fate			
Stream Description/Stream Component	Acute Toxicity	Genetic Point Mut.	Genetic Chrom.	Repeated Dose	Develop-mental	Reproduc-tion	Acute Fish	Acute Inverte-Brate	Algal Toxicity		Photo-deg.	Hydro-lysis	Fugacity	Biodeg-radation
Biphenyl feedstock	√	√	√					√						√
Aromatic pyrolysis oil	√	√	√											
Rerun tower bottoms	√	√	√	√	√									
Light pyrolysis fuel oil	√	√	√	√										
Pyrolysis fuel oil				√										
EDS experimental fuel oils (ETP 19 and 70/30)	√	√		√	√	√								
Heavy coker gas oil	√	√	√	√	√									

Table 5. Assessment Plan for Streams in Fuel Oils Category Under the Program.

	Human Health Effects						Environmental Toxicity			Physical Chem.	Environmental Fate			
Stream Description/Stream Component	Acute Toxicity	Genetic Point Mut.	Genetic Chrom.	Repeated Dose	Develop-mental	Reproduc-tion	Acute Fish	Acute Inverte-Brate	Algal Toxicity		Photo-deg.	Hydro-lysis	Fugacity	Biodeg-radation
Light fuel oil (typical)	RA	RA	RA	RA	RA	RA	T	T	T	T/SAR	TD	TD	CM	T
Heavy fuel oil (typical)	RA	RA	RA	RA	RA	RA	T	T	T	T/SAR	TD	TD	CM	T

A Adequate existing data available
CM Computer modeling proposed
NA Not applicable
TD Technical discussion proposed
T Testing proposed
RA Read-Across to existing data (see table 4)
SAR Structure-Activity-Relationship modeling and readily available data

Table 6. Aquatic Toxicity Data by Endpoint and Carbon Number(s) for a Fuel Oil Product, and Chemicals and Chemically Complex Products with Chemical Constituents that can be Found in Fuel Oils Category Products

TEST ORGANISM & ENDPOINT	CHEMICAL OR COMPLEX PRODUCT BY CARBON NUMBER(S)*[1]							
	C8 o-X[2]	**C8 m-X**[2]	**C8 p-X**[2]	**C9 EB**[3]	**C9 AB**[4]	**C10 N**[5]	**C10/12 N/AN**[6]	**C10/12 BP**[7]
Fish 96-hr LC_{50} (mg/L)	16.4 Fm	-	2.6 Rt	12.1 Fm	-	-	-	-
Daphnid EC_{50} (mg/L)	1.0 *Dm* [a]	4.7 *Dm* [a]	-	-	-	16.7 *Dm* [b]	-	-
Fish 96-hr LL_{50} (mg/L)	-	- -	- -	- -	18.0 Rt	-	3.0 Rt	-
Daphnid 48-hr EL_{50} (mg/L)	-	-	-	-	21.3 *Dm*	-	1.1 *Dm*	23.6 *Dm*

* Robust summaries for all materials except the biphenyl feedstock product (BP) are from the International Hydrocarbon Solvents Consortium and will be contained in selected SIARs (to be submitted)
1 Predominant carbon number(s) for complex products
2 Xylene (chemical)
3 Ethylbenzene (chemical)
4 Alkylbenzenes (complex product, predominantly C9)
5 Naphthalene (chemical)
6 Naphthalene / Alkylnaphthalenes (complex product, predominantly C10/11)
7 Biphenyl Feedstock (complex fuel oil product, predominantly C10-12 hydrocarbons)

LC/EC Lethal/Effect concentration
LL / EL Lethal / Effect loading
Fm Fathead minnow
Rt Rainbow trout
Dm[a] *Daphnia magna* - 24 hr study
Dm[b] Daphnia magna - 48 hr study

Table 7. Olefins Panel Sponsored HPV Test Categories

Category Number	Category Description
1	Crude 1,3-Butadiene C4
2	Low 1,3-Butadiene C4
3	C5 Non-Cyclics
4	Propylene Streams (3) – Propylene sponsored through ICCA
5	High Benzene Naphthas
6	Low Benzene Naphthas
7, 8, 9	Resin Oils and Cyclodiene Dimer Concentrates
10	Fuel Oils

Appendix I

ETHYLENE PROCESS DESCRIPTION

A. The Ethylene Process

1. Steam Cracking

Steam cracking is the predominant process used to produce ethylene. Various hydrocarbon feedstocks are used in the production of ethylene by steam cracking, including ethane, propane, butane, and liquid petroleum fractions such as condensate, naphtha, and gas oils. The feedstocks are normally saturated hydrocarbons but may contain minor amounts of unsaturated hydrocarbons. These feedstocks are charged to the coils of a cracking furnace. Heat is transferred through the metal walls of the coils to the feedstock from hot flue gas, which is generated by combustion of fuels in the furnace firebox. The outlet of the cracking coil is usually maintained at relatively low pressure in order to obtain good yields to the desired products. Steam is also added to the coil and serves as a diluent to improve yields and to control coke formation. This step of the ethylene process is commonly referred to as "steam cracking" or simply "cracking" and the furnaces are frequently referred to as "crackers".

Subjecting the feedstocks to high temperatures in this manner results in the partial conversion of the feedstock to olefins. In the simplest example, feedstock ethane is partially converted to ethylene and hydrogen. Similarly, propane, butane, or the hydrocarbon compounds that are associated with the liquid feedstocks are also converted to ethylene. Other valuable hydrocarbon products are also formed, including other olefins, diolefins, aromatics, paraffins, and lesser amounts of acetylenes. These other hydrocarbon products include compounds with two or more carbon atoms per molecule, i.e., C2, C3, C4, etc. Propane and propylene are examples of C3 hydrocarbons and benzene, hexene, and cyclohexane are a few examples of the C6 hydrocarbons.

2. Refinery Gas Separation

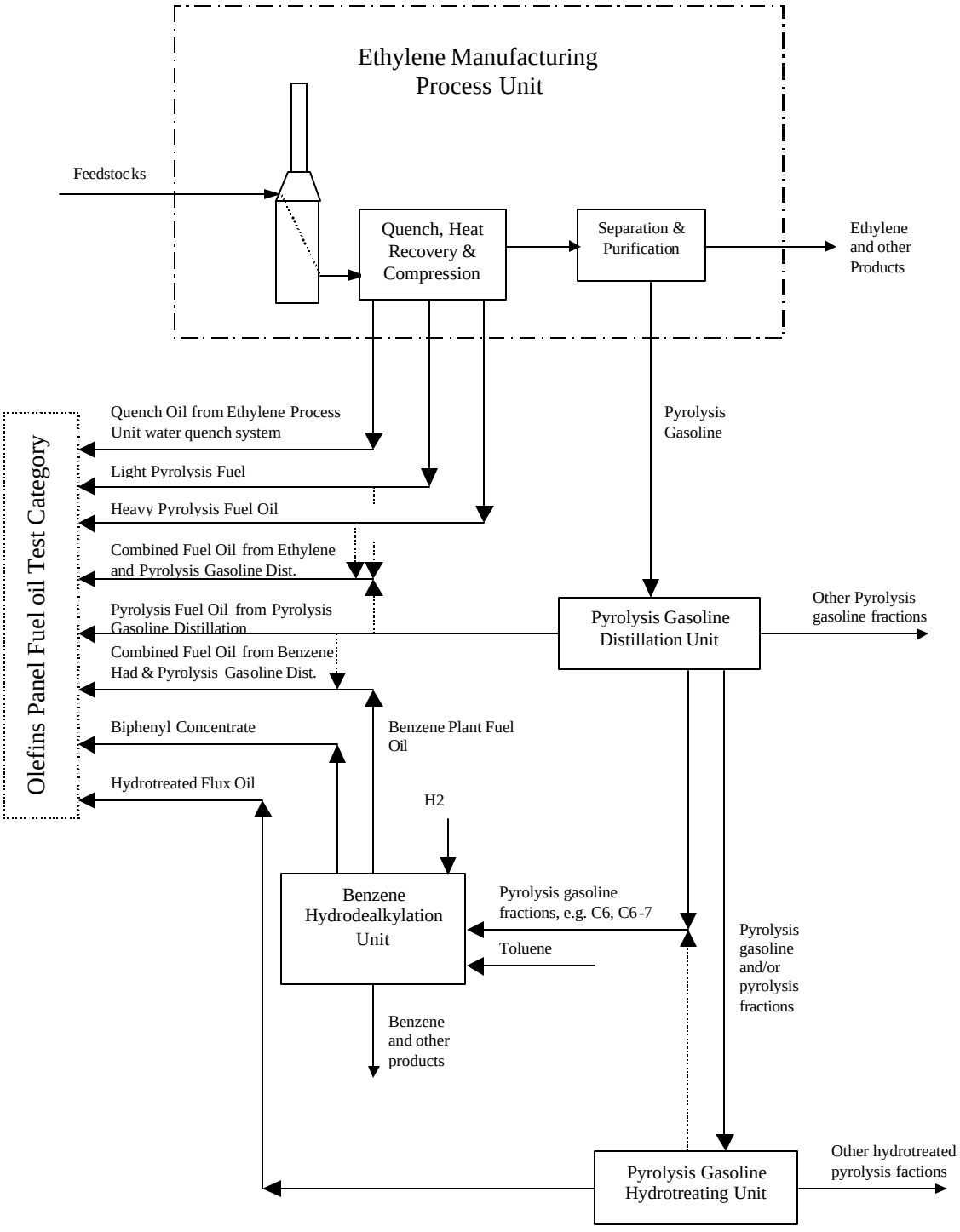
Ethylene and propylene are also produced by separation of these olefins streams, such as from the light ends product of a catalytic cracking process. This separation is similar to that used in steam crackers, and in some cases both refinery gas streams and steam cracking furnace effluents are combined and processed in a single finishing section. These refinery gas streams differ from cracked gas in that the refinery streams have a much narrower carbon number distribution, predominantly C2 and/or C3. Thus the finishing of these refinery gas streams yields primary ethylene and ethane, and/or propylene and propane.

B. Products of the Ethylene Process

The intermediate stream that exits the cracking furnaces (i.e., the furnace effluent) is forwarded to the finishing section of the ethylene plant. The furnace effluent is commonly referred to as "cracked gas" and consists of a mixture of hydrogen, methane, and various hydrocarbon compounds with two or more carbon atoms per molecule (C2+). The relative amount of each component in the cracked gas varies depending on what feedstocks are cracked and cracking process variables. Cracked gas may also contain relatively small concentrations of organic sulfur compounds that were present as impurities in the feedstock or were added to the feedstock to control coke formation. The cracked gas stream is cooled, compressed and then separated into the individual streams of the ethylene process. These streams can be sold commercially and/or put into further steps of the process to produce additional materials. In some ethylene processes, a liquid fuel oil product is produced when the cracked gas is initially cooled. The ethylene process is a closed process and the products are contained in pressure systems.

The final products of the ethylene process include hydrogen, methane (frequently used as fuel), and the high purity products ethylene and propylene. Other products of the ethylene process are typically mixed streams that are isolated by distillation according to boiling point ranges. It is a subset of these mixed streams that make up the constituents of the Fuel Oils Category. See Figure 1 for a schematic.

Figure 1
Chemical Process Operations Associated With Process Streams in the Fuel Oils Category



AR201-13435B

Robust Summary - Group 10: Fuel Oils

Acute Toxicity

Test Substance	Aromatic Pyrolysis Oil, CAS# 64742-90-1. A brown, tarry, highly viscous mixture
Method	
Method/guideline followed	No guidelines specified; comparable to standard study
Type (test type)	Acute limit test
GLP	Yes
Year	1982
Species/Strain	Rat, Fischer 344
Sex	Male and female
No. of animals per sex per dose	5
Vehicle	None
Route of administration	Oral gavage
Test Conditions	Rats (50 days old) were dosed once with undiluted aromatic pyrolysis oil at 5 g/kg. Each rat was observed for clinical signs, mortality and morbundity at 1 and 4 hours after dosing, and daily thereafter for 14 days. Body weight was obtained at initiation and after 7 and 14 days post dosing. At study termination, all rats were sacrificed and gross necropsies performed.
Results	
LC_{50} with confidence limits.	No mortality occurred during the study. Clinical signs included oily staining around the mouth and yellow staining of the inguinal region. No remarkable findings related to test article administration were observed at necropsy.
Remarks	
Conclusions	Acute oral median lethal dose for aromatic pyrolysis oil was not reached at a dose of 5 g/kg
Data Quality	**1.** Reliable without restrictions.
Reliability	
References	Rausina, G.A. 1982. Acute oral toxicity test in albino rats using aromatic pyrolysis oil. Proj. #82-114. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX.
Other	
Last changed	7/27/2001 (Prepared by a contractor to the Olefins Panel)

Acute Toxicity

Test Substance	Aromatic Pyrolysis Oil, CAS #64742-90-1. A brown, tarry, highly viscous mixture.
Method	
Method/guideline followed	No guidelines specified, comparable to standard study.
Type (test type)	Acute limit test
GLP	Yes
Year	1982
Species/Strain	Rat, Fischer 344
Sex	Male and female
No. of animals per sex per dose	5
Vehicle	Filtered air
Route of administration	Whole body inhalation
Test Conditions	One group of 10 individually housed rats (5m, 5F; 16-17wks old) was exposed to the aerosolized test substance for 4 hours in stainless steel dynamic exposure chambers, followed by 14 days of post exposure observation for clinical signs, morbidity and death. Body weight was taken before exposure, and 7 and 14 days post exposure. Nominal chamber concentration was 26.8g/m^3 (uncorrected for large particle condensation) and actual concentration was 3.7 g/m^3 as determined by fluorescence.
Results	
LC_{50} with confidence limits.	LC50 was not reached at the dose of 3.7 g/m^3.
Remarks	During exposure, males and females showed excessive tearing and nasal discharge that were absent by day 2 post-exposure. At day 2 and persisting for several days were observations of porphyria around the eyes and ocular discharge. At day 14, the only clinical findings were hair loss and hair discoloration. There were no changes in body weight gain, no deaths, and no abnormal findings at necropsy.
Conclusions	LC50 was not reached at 3.7 g/m^3.
Data Quality	
Reliability	**1.** Reliable without restrictions
References	Gordon, T. 1982. LC50 Aromatic Pyrolysis Oil inhalation study in rats. Proj. #82-082. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	7/27/2001 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vitro

Test Substance	Aromatic Pyrolysis Oil, Gulf. CAS #64742-90-1. Brown-black, highly viscous tarry residue. Composition analysis, purity and stability referred to sponsor.
Test substance	
Method	
Method/guideline followed	Standard method based on Hsie et al. (1981), O'Neill & Hsie (1979)
Type	In vitro mammalian cell forward mutation
System of testing	Chinese hamster ovary (CHO) cell culture
GLP	Yes
Year	1984
Species/Strain	CHO-K-1 heterozygous for hypoxanthine-guanine phosphoribosyl transferase (HGPRT+/-) from Oak Ridge National Laboratory, TN.
Metabolic activation	Yes
Species and cell type	Rat liver (S9) fraction purchased from Litton Bionetics, Kensington, MD
Quantity	1.0mg S9 fraction/ml treatment medium (0.3ml S9 fraction in 3 ml medium/flask)
Induced or not induced	Aroclor 1254 induced (treatment not specified)
Concentrations tested	Cytotoxicity: 8, 16, 32, 64, 128, 256, 512, 1023, 2048, 5000µg/ml ± S9; Mutagenicity: 32, 64, 96, 128, 175, 256µg/ml –S9; 128, 175, 256, 375, 512, 750µg/ml +S9; repeat : 500, 600, 750 µg/ml + S9 all diluted in 50% Pluronic® polyol F127 (prepared in absolute ethanol, mol. wt. 12,500).
Statistical Methods	Frequency of mutant colonies per million clonable cells was calculated and comparisons of treated cultures with vehicle controls made on transformed data using a two-tailed t-test (Irr & Snee, 1979). Criteria for positive results were significant ($p<0.05$) increase in mutant colonies (HGPRT+/-? HGPRT-/-) at any dose level and a dose related response. If only one criterion is met, results are considered equivocal.
Remarks for Test Conditions	Sufficient Aromatic Pyrolysis Oil (APO) was weighed separately for each dose level into 10 ml graduated vials; aliquots were kept under an inert nitrogen blanket overnight. 2.5ml of 50% F127 was added to each treatment vial and vehicle control and medium (Ham's F-12 without hypoxanthine) added as required to achieve final 10ml volume for testing. All dosing preparations were thoroughly blended with mixing rods to produce a uniform emulsion, kept in a water bath at 37^0C until dosing and mixed again just prior to use when 20µl of each preparation was added to 3ml treatment medium/culture vessel. All cultures were incubated at 37^0C in 5% CO_2 enriched, humidified atmosphere. Positive control mutagens were ethyl methanesulfonate (100µg/ml) for –S9 cultures, and benzo(a)pyrene (4µg/ml) for +S9 cultures. For cytotoxicity, each dose group was composed of 2 flasks, one –S9, one+S9, negative controls ± S9, seeded with $5x10^5$ cells on day 1. Cultures were exposed to test compound for 5 hours on day 2. On day 3, cells were trypsinized and counted with a Coulter Model ZB, then 200 cells were transferred into each of 3 60mm culture dishes. These viability plates were incubated until day 10, fixed in methanol and stained with Giemsa. Colonies were counted visually or with an Artek Model 981 colony counter. Absolute survival = total colony count ÷ number of cells seeded/flask. Relative survival = absolute survival in treated cultures ÷ vehicle control survival. Acceptable survival level is at least 10%. For mutagenicity, cells were seeded on day 1 into 6 flasks/dose group, 3-S9, 3+S9; on day 2 approximately 10^6 cells were exposed to APO for 5 hours. Vehicle control had 12 flasks, 6-S9, 6+S9. On day 3, cultures with excessive cytotoxicity were discarded. From remaining cultures, 200 cells were seeded to each of 4 viability plates/dose level; incubated to day 10, fixed with methanol, stained with Giemsa, and colonies counted for survival. Expression cultures (10^5-10^6 cells/one dish/dose) were seeded on day 3; subcultured three times until day 10 when 200 cells were seeded on each of 4 viability plates/dose and $2x10^5$ cells seeded on each of 5 mutagenicity plates/dose with selective medium containing 10^{-5}M 6-thioguanine to allow expression of HGPRT mutation. Cultures were incubated undisturbed until day 17-18 when they were fixed and stained. For mutagenicity , a ratio of total colony counts in mutagenicity plates over absolute survival in viability plates was calculated for each treatment group. Frequency of mutant

	colonies/million clonable cells was calculated and statistical comparisons with negative control data were made.
Results Genotoxic effects	In the cytotoxicity test, APO induced cell toxicity beginning at 32µg/ml +S9 (cells $x10^5$/ml 5.0, 4.3, 3.8, 3.9, 2.5 and 2.3 at 0[vehicle control], 32, 64, 128, 256 and 512µg/ml) and at 256µg/ml –S9 (cells $x10^5$/ml 6.2, 6.5, 5.4, 6.4, 0.5 and 2.2 at 0[vehicle], 32, 64, 128, 256 and 512µg/ml). Colony counts after treatment in –S9 cultures (cloned up to 128µg/ml) had no toxicity at any dose level, but +S9 cultures showed significant cytotoxicity at 256µg/ml and higher (relative survival 84.9 and 44.2 at 256 and 512µg/ml). In the mutagenicity test, post treatment reduced cell count was seen at all dose levels ± S9. Addition of S9 to APO treated cultures caused significant toxicity in colony counts at all dose levels (relative survival 64.9, 42.8, 35.4 and 13.6% at 256, 375, 512, 750µg/ml); no significant clonal cytotoxicity seen in –S9 cultures but cloning efficiency was low. (approx. 70 - 75%). Cloning efficiency was impaired at the time of mutant selection in +S9 cultures at 375µg/ml and higher (62.0, 65.9and 58.6% at 375, 512 and 750µg/ml). A significant increase in mutant frequency compared to vehicle controls was seen at 750µg/ml +S9 cultures accompanied by a somewhat linear dose related response at all dose levels of APO after metabolic activation (mutants/10^6 clonable cells 10.9, 25.4, 32.2, 14.5 and 49.8 at 0[vehicle] 256, 375, 512 and 750µg/ml). No mutagenic effects were observed in non-activated (-S9) cultures. Positive control compounds demonstrated appropriate responses (EMS-S9 214.5 mutants/10^6cells, B(a)P +S9 111 mutants/10^6 cells). In a repeat trial of the activated portion of the assay, APO induced a significant increase in mutant frequency at 500µg/ml, while higher doses of 600 and 750µg/ml were too toxic to be selected for cloning. A linear dose response was also observed over the clonable dose range (18.0, 30.8, 97.6 and 436.8 mutants/10^6 cells at 0, 250, 400 and 500µg/ml. These results confirmed the previous positive findings for APO in S9 activated cultures.
Conclusions (contractor)	Mutagenic effects of Aromatic Pyrolysis Oil were observed in cultures with metabolic activation at a dose of 750ug/ml (1^{st} trial) and at 500ug/ml (2^{nd} trial) accompanied by linear dose related responses in both trials. Aromatic Pyrolysis Oil induces gene point mutations in the presence of rat liver metabolic activation in CHO/HGPRT cells under conditions of this assay.
Data Quality *Reliabilities*	**1.** Reliable without restrictions.
Reference	Papciak, M.S., Goode, J.W. 1984. CHO/HGPRT test using Aromatic Pyrolysis Oil. Proj. #2081. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Hsie, A.W. et al. 1981. Mut. Res. 86: 193-214 O'Neill, J.P. and Hsie, A.W. 1979. Banbury Report 2: 55-63 Irr, J.D. and Snee, R.D. 1979. Banbury Report 2: 263-275.
Other *Last changed*	Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vitro

Test Substance	Aromatic Pyrolysis Oil, Gulf. CAS #64742-90-1. brown-black, highly viscous tarry residue. Composition analysis, purity and stability referred to sponsor.
Test substance	
Method	
Method/guideline followed	Standard method based on Williams et al. (1977,1982)
Type	In vitro mammalian cell DNA repair assay
System of testing	Unscheduled DNA Synthesis (UDS) in primary hepatocyte cultures.
GLP	Yes
Year	1984
Species/Strain	Fischer 344 male rat (9-10 wks old) – 1 rat per test
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Range-finding: 4, 8, 16, 32, 64, 128, 256, 512, 1024 µg/ml: UDS assay 0.5, 2.0, 10, 60µg/ml; all diluted in 50% Pluronic® polyol F127 (prepared in absolute ethanol, mol. wt 12,000, 70% hydrophilic)
Exposure period	18-20 hours
Statistical Methods	None employed. Criteria for positive response are incorporation of radioactive precursor (^{3}H-thymidine) in cells that are not normally synthesizing DNA, indicating repair of damage. A positive response is defined as a mean net nuclear grain count at any treatment level that exceeds concurrent negative control by at least 6 grains/nucleus; negative control value must not exceed 5 grains. A positive response need not be dose related.
Remarks for Test Conditions	Sufficient Aromatic Pyrolysis Oil (APO) was weighed separately for each dose level, 0.75ml of 50% F127 added per ml of final volume and sufficient medium (Williams Medium E with 10% fetal bovine serum and insulin) added to achieve final volume. Test preparations were mixed just prior to addition at 30 or 50µl to each 3 or 5ml culture, respectively. The conc. of ^{3}H-thymidine (½ life 12.5 yrs.) used in these assays was 1mCi/ml. All cultures were incubated at 37^0C in 5% CO_2 enriched humidified atmosphere. For range-finding, primary hepatocytes derived from freshly perfused rat liver were seeded (approx. $1x10^5$ cells/ml) into treatment vessels, exposed to test material for 18 hours (2 cultures/dose level; 2 untreated cultures, and two vehicle F127 control cultures), then fixed in formalin and stained with trypan blue for viability determination. At least 50% viability needed for the assay. In the UDS assay, $1x10^5$ cells/ml were seeded into coverslip cultures, exposed to ^{3}H-thymidine and test substance for 18 hours (3 cultures/dose level). Positive control was 2-acetyl aminofluorene (0.2µg/ml). Cells growing on coverslips were rinsed, fixed and glued to microscope slides on day 2. On day 3, slides were dipped in autoradiographic emulsion and stored in the dark at $2\text{-}8^0$C. Autoradiographs were developed, stained and coverslipped on day 14. Number of grains overlying 50 randomly selected nuclei/slide were counted. The highest of 3 cytoplasmic grain counts/cell were subtracted to obtain net nuclear grain count. Avg. net nuclear grain count/slide (sum of net nuclear grain count ÷ 50) and mean net nuclear grain count (avg. net nuclear grain count/slide ÷3) were calculated.
Results	APO induced toxicity in primary hepatocytes beginning at 4µg/ml (51.2% viability) following 18-20 hours exposure. Viability continued to decrease in a generally dose related manner to the maximum dose of 1024 µg/ml (0.8% relative viability). UDS occurred in a dose related manner, increasing from 117 net nuclear grains at 2 µg/ml to 218 grains at 60 µg/ml compared to a vehicle control net count of 0.63 and positive control of 363 net nuclear grains.
Genotoxic effects	
Conclusions	Aromatic Pyrolysis Oil induced dose related unscheduled DNA synthesis in cultured rat hepatocytes. Aromatic Pyrolysis Oil causes DNA damage and repair in this assay.
(contractor)	

Data Quality *Reliabilities*	**1**. Reliable without restrictions. Study conforms to standard design. GLPs have been followed.
Reference	Brecher, S., Goode, J.W. 1984. Hepatocyte primary culture/DNA repair test of aromatic pyrolysis oil. Proj. #2083. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Williams, G.M. 1977. Cancer Res. 37: 1845-1851 Williams et al. 1977. In Vitro 13: 809-817 Williams et al. 1982. Mut. Res. 97:359-370
Other *Last changed*	4/11/2001 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vitro

Test Substance	Aromatic Pyrolysis Oil, Gulf. CAS #64742-90-1. Dark brown-black, highly viscous tarry residue. Composition analysis, purity and stability referred to sponsor.
Test substance	
Method	
Method/guideline followed	Standard method based on Cortesi et al (1983), Dunkel et al (1981), Reznikoff et al (1973)
Type	In vitro cell transformation
System of testing	Mouse embryo cells
GLP	Yes
Year	1983
Species/Strain	BALB/3T3-A31-1-1 from T. Kakunaga, National Cancer Inst., 1982
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Cytotoxicity: 4, 8, 16, 32, 64, 128, 256, 512, 1024 µg/ml; Transformation: 8, 16, 32, 64, 128, 256 µg/ml, all diluted in 50% Pluronic® polyol F127 (prepared in absolute ethanol, mol. wt. 12,000, 70% hydrophilic).
Exposure period	2 days
Statistical Methods	None employed. Criteria for positive response were a two-fold increase in type III foci at the highest dose over vehicle control (at least 2 type III foci if vehicle control had none) with or without a dose related response, or a two-fold increase at two or more consecutive doses. Test is equivocal if two-fold increase occurred at any one level other than the highest acceptable dose.
Remarks for Test Conditions	Sufficient Aromatic Pyrolysis Oil (APO) was weighed separately for each dose level, 0.75ml of 50% F127 added per ml of final volume and medium (Eagles MEM with 10% heat-inactivated fetal calf serum) added as required to achieve final volume for testing. Test preparations were mixed just prior to addition to cultures at 50µl to each 5 ml culture. All cultures were incubated at 37^0C in 5% CO_2 enriched humidified atmosphere. For cytotoxicity, 2 plate cultures/dose group, 2 plate cultures for vehicle F127 or medium negative control were seeded with $1x10^4$ cells/plate in day 1, exposed on days 2-3, trypsinized and counted with a Coulter Model ZB on day 4 for at least 20% survival. For transformation, 15 flasks ($1x10^4$ cells/flask/dose group)) and two plate cultures (100 cells/plate/dose group) were seeded on day 1, exposed on days 2-3 and culture medium changed on day 4. For flask cultures, medium continued to be changed weekly to day 29. Positive control was 3-methylcholanthrene (1ug/ml). Plate cultures were fixed, stained, and counted visually on day 9 to determine cloning efficiency (avg. number colonies/plate ÷ 100 cells seeded). Flask cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci ÷ total flasks/dose group.
Results	APO induced toxicity in BALB/3T3 cells after two days exposure beginning at 128 µg/ml (59% relative survival); maximum toxicity (80%) occurred between 256-512 µg/ml and plateaued at 1024 µg/ml. APO induced transformed foci at 128, 256 µg/ml, the two highest dose levels, with borderline positives but inconsistent responses at 8-64 µg/ml. Compounds that transform cells have a high probability of inducing tumors if injected into immunosuppressed mice. Positive and negative controls gave expected responses.
Genotoxic effects	
Conclusions (contractor)	Aromatic Pyrolysis Oil induced transformation in BALB/3T3 cells under conditions of this assay. Cytotoxicity and impairment of cloning efficiency were also observed at the two highest dose levels.
Data Quality *Reliabilities*	**1**. Reliable without restriction.
Reference	Brecher, S., Good, J.W. 1983. BALB/3T3 transformation test: Aromatic Pyrolysis Oil. Proj. #2084. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil

	Pyrolysis Oil. Proj. #2084. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co, Houston, TX Cortesi, E. et al. 1983. Teratogenesis, Carcinogenesis, Mutagenesis 3: 101-110. Dunkel, V.A. et al. 1981. J. Nat'l Cancer Inst. 67: 1303-1315. Reznikoff, C.A. et al. 1973. Cancer Res. 3239-3249.
Other *Last changed*	7/27/2001 (Prepared by a contractor to the Olefins Panel).

Genetic Toxicity - in Vivo

Test Substance	
Remarks	Aromatic Pyrolysis Oil, Gulf CAS #64742-90-1 Dark brown - black, highly viscous residue. Compositional analysis, purity and stability referred to sponsor.
Method	
Method/guideline followed	Comparable to standard assay
Type	Mammalian bone marrow erythrocyte micronucleus
GLP	Yes
Year	1984
Species	Mouse
Strain	Crl:CD®–1 (ICR) BR Swiss
Sex	Male and female: 10M, 10F/group; 15M, 15 F in 1 group (11wks old at intiation)
Route of administration	Oral gavage
Doses/concentration levels	0.0, 1.25, 2.5, 5.0 g/kg in corn oil
Exposure period	1 dose/day for 2 days; 1 group- 1 dose, 1 day only
Statistical methods	Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant ($p<0.05$) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met.
Remarks for Test Conditions.	Aromatic pyrolysis oil (APO) dosing solutions were prepared fresh for each day of dosing –12.5 g APO mixed with corn oil to make 50 ml, blended by shaking. Three groups of mice were given APO by oral gavage daily for two days. All mice were weighed on day 1 and on day of sacrifice. One half of each treated group and vehicle control (5M, 5F) was killed on day 3 and the remainder on day 4. One group (15M, 15F), given 5.0 g/kg by gavage in a single dose for 1 day only, was killed on days 2, 3, 4 (5/sex/day). Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of femoral bone marrow smears were prepared, stained with May-Grunewald/Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all associated mature erythrocytes (NORMs) were counted. Data collected included group mean body weights for each day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN.
Results	
Genotoxic effects	Males at all dose levels treated with 1.25-5.0 g/kg APO for 2 days showed statistically significant ($p<0.05$) dose related increases in % micronucleated PCE compared to concurrent negative controls at both day 3 and day 4 sacrifices, except for the 1.25 g/kg group on day 4 which was significantly higher than the historical control only. Females given 2 doses and sacrificed on days 3 and 4 showed statistically significant ($p<0.05$) increases in % micronucleated PCE compared to negative controls at 5.0 g/kg only. All mice given 1 dose of 5.0 g/kg and sacrificed on day 2, 3, 4 showed positive responses compared to negative controls. There were no significant changes in the ratio of PCE/ NORM compared to controls. LOEL (males) = 1.25 g/kg: NOEL (females) = 2.5 g/kg
NOAEL (NOEL)	
LOAEL (LOEL)	
Conclusions	
(study authors)	Oral treatment of mice with Aromatic Pyrolysis Oil for 1 or 2 days induced increased frequency of micronucleated polychromatic erythrocytes in bone marrow and is considered positive. APO can induce cytogenetic damage in this test system.
Data Quality	
Reliabilities	**1.** Reliable without restrictions.
References	Khan, S.H. and Goode, J.W. 1984. Micronucleus test: Aromatic Pyrolysis Oil orally for 2 days. Proj. #2082. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	7/27/2001 (Prepared by a consultant to the Olefins Panel)

Repeated Dose Toxicity

Test Substance	Aromatic Pyrolysis Oil, CAS #64742-90-1. Black tarry material. Refer to sponsor for analysis of composition and purity.
Remarks	
Method	
Method/guideline followed	No guidelines specified, comparable to standard study.
Test type	Subacute
GLP	Yes
Year	1983
Species	Rat
Strain	Fischer 344
Route of administration	Dermal
Duration of test	2 weeks
Doses/concentration levels	0, 1, 2 g/kg
Sex	Males and females (5/sex/group), 7 wks old at initiation of study
Exposure period	6 hr./day for 9 days over a 14 day period
Frequency of treatment	once/day, 5 days/week
Control group and treatment	corn oil, 1.82 ml/kg
Post exposure observation period	None
Statistical methods	Bartlett's test, Analysis of Variance, Dunnett's test, Kolmogorov-Smirnov two-tailed test
Test Conditions	Animals were housed individually in suspended stainless steel cages with wire mesh bottoms and fronts in a room maintained at 69.8^0F with relative humidity of 46.5% and 12 hour light/dark cycle. Water and chow diet were provided ad lib. Doses of test article were administered over 10% of body surface to the backs of rats clipped free of hair and fitted with Elizabethan collars to reduce ingestion. After 6 hours, collars were removed and residual oil wiped off. Observations for mortality and morbidity were made twice/day on dosing days and once/day on non-dosing days, and for clinical signs at least once daily. Food consumption was determined weekly and body weight at study initiation and weekly thereafter. Dermal reactions were scored on each dosing day, before and after exposure. At sacrifice, gross necropsy was performed and liver,brain, spleen, heart, kidneys and testes were weighed. Slides of sections of the weighed organs, and also ovaries, uterus and skin were prepared for histopathologic examination of control and high dose groups.
Results	
NOAEL (NOEL)	NOEL not determined. LOEL = 1.0 g/kg
LOAEL (LOEL)	
Remarks	No deaths or moribund rats were observed. Food consumption was decreased in all test article dosed rats; in males the decrease was dose related. In both males and females body weight was reduced in a dose related manner. Dermal effects were difficult to evaluate because of the black/tarry test material; however, in the high dose group, after a weekend without dosing, erythema was moderate to severe with fissuring and skin peeling. Skin histopathologic effects consisted of moderate to marked acanthosis and hyperplasia of the epithelium, and hyperkeratosis. No other test article related histopathologic lesions were found. There were no statistically significant changes in clinical chemistry and hematology parameters. Elevated absolute and relative liver weight was observed in all treated groups.
Conclusions	Aromatic pyrolysis oil at 1.0 and 2.0 g/kg caused depression of body weight gain associated with decreased food consumption. At 2.0 g/kg, all rats showed moderate to severe erythema (Draize score 3-4). Fissures and peeling skin were seen at 2.0 g/kg but not at 1.0 g/kg. The 1.0 g/kg animals were not scored for erythema. Skin changes consisted of hyperplasia and acanthosis.
Quality	
Reliabilities	**1.** Reliable without restrictions
References	Zellers, J.E. 1983. Two week repeated dose toxicity study in rats using aromatic pyrolysis oil. Proj. #82-089. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	4/11/2001 (Prepared by a contractor to the Olefins Panel)

Repeated Dose Toxicity

Test Substance	Aromatic Pyrolysis Oil, CAS #64742-90-1. Dark brown to black, highly viscous, tarry residue
Remarks	
Method	
Method/guideline followed	None specified; comparable to standard study.
Test type	Subacute
GLP	Yes
Year	1983
Species	Rat
Strain	Fischer 344
Route of administration	Whole body inhalation
Duration of test	12 days
Doses/concentration levels	0, 0.54, 2.00 g/m^3 (actual)
Sex	Male and female (5M, 5F/group)
Exposure period	9 days
Frequency of treatment	6 hr/day
Control group and treatment	filtered air, 6 hr/day for 9 days
Post exposure observation period	2 days
Statistical methods	Analysis of variance, Dunnett's test, Kolmogorov-Smirnov two tailed test.
Test Conditions	Animals (17 wks old) were housed individually and exposed to the test material for 6 hr/day in stainless steel dynamic exposure chambers. Temperature was maintained at 77^0F and relative humidity at 45%. Water and chow diet were provided ad lib. Nominal concentrations were measured gravimetrically and actual concentrations by fluorescence. Rats were observed twice daily on dosing days and once daily on non-dosing days for morbidity and mortality, and immediately after exposure for clinical signs. Body weight was taken at initiation, day 5 of treatment, and prior to sacrifice (day 12) after 2 days of recovery. Blood was collected via orbital sinus on day 12 for hematology and clinical chemistry. At sacrifice, necropsies were performed and target organs were weighed and preserved for histopathology.
Results	NOEL not determined
NOAEL (NOEL)	LOEL = 0.54 g/m^3
LOAEL (LOEL)	
Remarks	Males and females showed dose and time related decreases in body weight that were more severe in the male. Males and females showed dose related increases in clinical symptoms (hair loss, nasal discharge, discharge from eyes, eyes closed and perianal soiling). At the high dose, one female showed arching of the back. Clinical pathology values were unremarkable. Treated male and female rats, showed yellow discoloration of the lungs grossly and hyperplasia of the pulmonary alveolar macrophages microscopically. The organ weights of the high dose males' and females' livers, the high dose females' lungs and the low dose females' livers were significantly increased relative to control animal values. The splenic weights of the high dose male and female rats were significantly decreased. A decrease was observed in the high dose male heart weights compared to control male data.
Conclusions	No mortality or morbidity was observed. Exposure related signs included weight loss, hair loss, nasal and ocular discharge and arched walking. Skin irritation was observed at both dose levels, which resolved after the 2 days recovery period in the 0.54 g/kg dose group. There were several alterations in absolute and relative organ weights in both sexes at both test article doses. Frequency and severity of effects were related to exposure level.
Quality	**2.** Reliable with restrictions. The report is not well written, key parameters that should have been noted in the methods section are scattered in the report. Most effects were clearly dose related but this was not evaluated statistically.
Reliabilities	
References	Gordon, T. 1983. Nine-day repeated dose inhalation toxicity study in rats: Aromatic Pyrolysis Oil. Proj. #2035. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	7/27/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 10: Fuel Oils

Acute Toxicity

Test Substance	Biphenyl Feedstock, CAS #68989-41-3. Dark brown aromatic slurry consisting of crystalline aggregates in a liquid medium. Refer to sponsor for further information.
Method	
Method/guideline followed	None specified. Comparable to standard study.
Type (test type)	Acute
GLP	Yes
Year	1983
Species/Strain	Rat, Fischer 344
Sex	Males and females
No. of animals per sex per dose	5M, 5F
Vehicle	Corn oil
Route of administration	Oral
Test Conditions	Rats were individually housed in metal screen-bottomed cages, provided with chow diet and water ad lib and maintained at 72-78^{0}F, with relative humidity of 23-64% and 12 hour light/dark cycle. Doses for the single dose acute LD_{50} study were established by performing two preliminary range-finding studies and a more definitive limit test at 5 g/kg. This was followed by testing at 2.5, 3.0, 3.5 and 4.0g/kg. Test article was administered in the form of a 30% (w/v) suspension in corn oil. At study initiation, rats were 91 days old for the limit test and 63 days old for the LD_{50} determination. Rats were fasted for 24 hr before dosing, and were observed daily for 14 days after dosing for clinical signs, moribundity and mortality. Gross necropsies were performed on all rats. LD_{50} determinations were performed by the method of Litchfield and Wilcoxin.
Results	
LD_{50} with confidence limits.	LD_{50} (95% confidence limits), combined sexes = 3.7g/kg (3.3-4.2) LD_{50} males = 3.6g/kg (3.08-4.21); LD_{50} females = >2.5, <4.0g/kg
Remarks	All surviving rats gained weight normally after 7 and 14 days. Deaths/dose are as follows: Males – 2.5g/kg, 0/5; 3.0g/kg, 0/5; 3.5g/kg, 3/5; 4.0g/kg, 4/5; 5g/kg, 5/6. Females – 2.5g/kg, 0/5; 3.0g/kg, 3/5; 3.5 g/kg 0/5; 4.0g/kg, 3/5; 5g/kg, 5/5. Clinical signs included lethargy, excessive lacrimation, dark inguinal stains and diarrhea that persisted until death or diminished in the last several days of the study. At gross necropsy, in rats that died during the study, a black oily substance was usually found in the stomach and/or intestines; this finding was not observed in surviving animals necropsied after 14 days.
Conclusions (study author)	Acute oral LD_{50} = 3.7g/kg (95% conf. limits 3.3-4.2g/kg). A black oily substance was usually found in the stomach and/or intestines of rats dying during the study.
Data Quality	
Reliability	**1.** Reliable without restriction
References	Rausina, G.A. 1983. Acute oral toxicity study in albino rats, Biphenyl Feedstock. Proj. #2036. Gulf Life Sciences Center, Pittsburgh PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel)

Acute Toxicity

Test Substance	Biphenyl Feedstock, CAS #68989-41-3. Amber slurry with an aromatic odor. Refer to sponsor for further information.
Method	
Method/guideline followed	None specified. Comparable to standard study
Type (test type)	Acute limit test
GLP	Yes
Year	1983
Species/Strain	Rabbit, New Zealand White
Sex	Males and females
No. of animals per sex per dose	5M, 5F
Vehicle	None
Route of administration	Dermal
Test Conditions	Rabbits (3.1 –3.6 kg) were individually housed in suspended metal screen-bottomed cages, in a room maintained at 72-80^{0}F and relative humidity of 34-60%. Neat test material was applied to the shaved abraded skin on backs (4 parallel, lengthwise, epidermal abrasions) at 2g/kg, and the trunk was wrapped with occlusive sheeting. Each rabbit was fitted with an Elizabethan collar to minimize ingestion during the first 24 hrs of exposure, after which time, the collar and wrappings were removed and the test substance wiped off with a dry towel. Observations for mortality and moribundity, clinical signs, and local skin reactions were continued for 14 days. Body wt was recorded prior to testing and at 7 and 14 days. Gross necropsies were performed after sacrifice.
Results	
LD_{50} with confidence limits.	LD_{50} was not reached at a single dermal dose of 2g/kg. One treated female rabbit died on day 1 of the study; no clinical signs were observed before the death. Body weights remained stable throughout the study. The other 9 rabbits appeared normal throughout the 14-day observation period. A delayed skin reaction was observed on day 5 when a well-defined skin irritation was noted in all test rabbits. This irritation decreased during the remainder of the study. Desquamation of skin was found after day 8 in 8 of 9 rabbits. Gross necropsies on all rabbits showed no findings attributable to test article administration.
Remarks	
Conclusions (study author)	LD_{50} was not reached at a single dose of 2g/kg. The test article produced a delayed skin irritation that first appeared at day 5 and resolved by day 12.
Data Quality	
Reliability	**1.** Reliable without restrictions
References	Rausina, G.A. 1983. Acute dermal toxicity study in albino rabbits with Biphenyl feedstock. Proj. #2037. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel

Acute Toxicity

Test Substance	Biphenyl Feedstock, CAS #68989-41-3. Light brown and waxy as solid; amber color as liquid at 70^0C. Refer to sponsor for further information.
Method	
Method/guideline followed	None specified. Comparable to standard study.
Type (test type)	Acute limit test
GLP	Yes
Year	1982
Species/Strain	Rat, Fischer 344
Sex	Males and females
No. of animals per sex per dose	10 rats (5M, 5F)/group
Vehicle	filtered air
Route of administration	Inhalation
Test Conditions	One group of Fischer 344 rats was exposed to aerosolized test article at a concentration of 3.0g/m^3 (actual) for 4 hr on day 1, followed by 13 days of post-exposure observation for mortality, moribundity, and clinical signs of toxicity. Rats were sacrificed on day 14. Food and water were available ad lib. Rats weighed between 152g and 262g at initiation. Body wt was recorded prior to exposure and on days 7 and 14 (prior to sacrifice and necropsy). The test substance was aerosolized with a nebulizer. Melted test article (80^0C) was poured into the nebulizer maintained at approx. 75^0C during atmosphere generation. A condensing flask prevented large diameter particles from entering the exposure chamber. Actual chamber concentration was 3.0g/m^3 (TWA) that was primarily particulate with mass median aerodynamic diameter of 4.3 microns.
Results	LC_{50} was not reached at 3.0g/m^3.
LC_{50} with confidence limits.	No deaths occurred from the single 4 hr exposure to 3.0g/m^3, which was the maximum attainable concentration of test article. Immediately after exposure, all rats were covered with crystalline test article; nearly every rat had dry red material around nose and mouth, perianal soiling, clear ocular discharge, porphyrin around the eyes, and discolored fur. Two males and one female showed labored respiration. These symptoms subsided during the observation period, and at sacrifice, only discolored fur was seen. Body wt was unchanged. No test article related lesions were detected at necropsy.
Remarks	
Conclusions (study author)	LC_{50} was not reached at 3.0g/m^3 and no evidence of systemic toxicity was observed.
Data Quality	
Reliability	**1.** Reliable without restrictions
References	Gordan, T. 1982. Acute LC_{50} inhalation toxicity test in rats with Biphenyl feedstock. Study # 82-086. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	6/07/2001 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vitro

Test Substance	Biphenyl Feedstock, CAS #68989-41-3. Amber-colored liquid with aromatic odor.
Test substance	Compositional analysis, purity and stability referred to sponsor.
Method	
Method/guideline followed	Standard method based on Hsie et al. (1981), O'Neill & Hsie (1979)
Type	In vitro mammalian cell forward mutation
System of testing	Chinese hamster ovary (CHO) cell culture
GLP	Yes
Year	1984
Species/Strain	CHO-K-1 heterozygous for hypoxanthine-guanine phosphoribosyl transferase (HGPRT+/-) from Oak Ridge National Laboratory, TN.
Metabolic activation	Yes
Species and cell type	Rat liver (S9) fraction purchased from Litton Bionetics, Kensington, MD
Quantity	1.0mg S9 fraction/ml treatment medium
Induced or not induced	Aroclor 1254 induced (treatment not specified)
Concentrations tested	Cytotoxicity: 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048µg/ml ± S9; Mutagenicity: 4, 8, 16, 21, 26, 32, 64µg/ml ±S9; all diluted in 10% Pluronic® polyol F68 (prepared in deionized water, mol. wt. 8350).
Statistical Methods	Frequency of mutant colonies per million clonable cells, corrected for absolute survival by viability plates, was calculated and comparisons of treated cultures with vehicle controls made on transformed data using a two-tailed t-test (Irr & Snee, 1979) using the MUTANT computer program (Snee et al., 1981). Criteria for positive results were a significant ($p<0.05$) increase in mutant colonies (HGPRT+/-? HGPRT-/-) at any dose level and a dose related response. If only one criterion is met, results are considered equivocal.
Remarks for Test Conditions	Sufficient Biphenyl feedstock was weighed separately for each dose level into 10ml volumetric flasks and stored overnight. The following day, flasks were placed in a water bath heated to approx. 93^0C to melt the biphenyl feedstock, then 7.0ml of 10% F68 was added to the highest dose flask, and to each subsequent dose preparation and vehicle control flask along with sufficient medium (Ham's F-12 without hypoxanthine) to achieve final 10ml volume for testing. All flasks were replaced in the water bath. Dosing preparations were vortexed after addition of all components, and just prior to dosing when 20µl were added to each 3 ml culture. All cultures were incubated at 37^0C in 5% CO_2 enriched, humidified atmosphere. Positive control mutagens were ethyl methanesulfonate (100µg/ml) for –S9 cultures, and benzo(a)pyrene (4µg/ml) for +S9 cultures. For range finding (cytotoxicity), each dose group was composed of 2 flasks, one –S9, one+S9, negative controls ± S9, seeded with $5x10^5$ cells on day 1. Cultures were exposed to test compound for 5 hours on day 2. On day 3, cells were trypsinized and counted with a Coulter Model ZB, then 200 cells were transferred into each of 3 60mm culture dishes. These viability plates were incubated until day 10, fixed in methanol and stained with Giemsa. Colonies were counted visually or with an Artek Model 981 colony counter. Absolute survival = total colony count ÷ number of cells seeded/flask. Relative survival = absolute survival in treated cultures ÷ vehicle control survival. Acceptable survival level is at least 10%. For mutagenicity, cells were seeded on day 1 into 6 flasks/dose group, 3-S9, 3+S9; on day 2 approximately 10^6 cells were exposed to biphenyl feedstock for 5 hours. Vehicle control had 12 flasks, 6-S9, 6+S9. On day 3, cultures with excessive cytotoxicity were discarded. From remaining cultures, 200 cells were seeded to each of 4 viability plates/dose level; incubated to day 10, fixed with methanol, stained with Giemsa, and colonies counted for survival. Expression cultures (10^5-10^6 cells/one dish/dose) were seeded on day 3; subcultured three times until day 10 when 200 cells were seeded on each of 4 viability plates/dose and $2x10^5$ cells seeded on each of 5 mutagenicity plates/dose with selective medium containing 10^{-5}M 6-thioguanine to allow expression of HGPRT mutation. Cultures were incubated undisturbed until day 17 when they were fixed and stained. For mutagenicity, a ratio of total colony counts in mutagenicity plates over absolute survival in

	viability plates was calculated for each treatment group. Frequency of mutant colonies/million clonable cells was calculated and statistical comparisons with negative control data were made.
Results	
Genotoxic effects	In the cytotoxicity test, Biphenyl feedstock induced toxic effects at concentrations of 32 µg/ml and higher –S9, and at 16µg/ml and higher +S9. Relative survival after treatment –S9 was 96.2, 9.1, and 0.0% at 16, 32 and 64µg/ml, respectively, and +S9 was 74.2, 23.9, 17.3 and 0.0% at 16, 32, 64 and 128µg/ml respectively. Some outlier cytotoxicity was observed at 4µg/ml –S9 (81.2% relative survival). In the mutagenicity test, cell count toxicity was observed beginning at 8µg/ml ±S9; cytotoxicity in colony counts occurred at 16µg/ml and higher ±S9. Absolute survival was significantly decreased only in +S9 cultures at concentrations of 26µg/ml and higher. Relative survival after treatment was 59.3 and 13.2% at 16 and 21µg/ml –S9, and 59.8 and 34.5% at 16 and 64µg/ml+S9. . Number of mutants/10^6 clonable cells –S9 was 13.7, 21.8, 22.5, and 0.5 at 4, 8, 16 and 21µg/ml respectively compared to controls (vehicle 22.7, medium 24.4) and +S9 was 1.0, 12.7, 5.3 and 17.7 at 16, 26, 32, and 64µg/ml, respectively compared to controls (vehicle 8.0, medium 14.7). There was no statistically significant increase in mutant frequency for any dose level of biphenyl feedstock compared to controls ±S9 in this assay Positive controls (EMS-S9 192.4 mutants/10^6 clonable cells; B(a)P +S9 32.7 mutants/10^6 clonable cells) and negative controls demonstrated appropriate responses .
Conclusions	
(contractor)	Biphenyl feedstock did not induce a mutagenic response with or without metabolic activation in CHO/HGPRT cells at any dose level. Cytotoxic effects were observed in both activated and non-activated cultures, demonstrating interaction of the test material with the cell system. Biphenyl Feedstock does not cause gene point mutations under the conditions of this assay.
Data Quality	
Reliabilities	**1**. Reliable without restrictions.
Reference	Papciak, R.J. and Goode, J.W. 1984. CHO/HGPRT test: using Biphenyl Feedstock. Proj. #2076. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Hsie, A.W. et al. 1981. Mut. Res. 86: 193-214 O'Neill, J.P. and Hsie, A.W. 1979. Banbury Report 2: 55-63 Irr, J.D. and Snee, R.D. 1979. Banbury Report 2: 263-275. Snee, R.D., Smith, R.L., and Irr, J.D. 1981. MUTANT. A computer program for the evaluation of short-term mutation test results. E.I. Dupont de Nemours Co.
Other	
Last changed	Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vitro

Test Substance	Biphenyl Feedstock, CAS #68989-41-3. Amber liquid with aromatic odor. Composition analysis, purity and stability referred to sponsor.
Test substance	
Method	
Method/guideline followed	Standard method based on Williams et al. (1977,1982)
Type	In vitro mammalian cell DNA repair assay
System of testing	Unscheduled DNA Synthesis (UDS) in primary hepatocyte cultures.
GLP	Yes
Year	1984
Species/Strain	Fischer 344 male rat (11-12 wks old) – 1 rat per test
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Range-finding: 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048µg/ml: UDS assay 5.0, 20.0, 50.0, 100µg/ml; all diluted in 10% Pluronic® polyol F68 (prepared in deionized water, mol. wt 8350, 80% hydrophilic)
Exposure period	18.25-18.5 hours
Statistical Methods	None employed. Criteria for positive response are incorporation of radioactive precursor (^{3}H-thymidine) in cells that are not normally synthesizing DNA, indicating repair of damage. A positive response is defined as a mean net nuclear grain count at any treatment level that exceeds concurrent negative control by at least 6 grains/nucleus; negative control value must not exceed 5 grains. A positive response need not be dose related.
Remarks for Test Conditions	Sufficient Biphenyl feedstock was weighed separately for each dose level and melted in an 85^{0}C water bath; 0.78ml of 10% F68 added per ml of final volume with sufficient balanced salt solution added to achieve final volume. Test preparations were stored at 50-60^{0}C until dosing when preparations were mixed and 30µl added to each 3ml culture. The conc. of ^{3}H-thymidine (½ life 12.5 yrs.) used in these assays was 1mCi/ml. All cultures were incubated at 37^{0}C in 5% CO_2 enriched humidified atmosphere. For range-finding, primary hepatocytes derived from freshly perfused rat liver were seeded (approx. 1x10^5 cells/ml) into treatment vessels, exposed to test material for 18.5 hours (2 cultures/dose level; 2 untreated cultures, and two vehicle F68 control cultures), then fixed in formalin and stained with trypan blue for viability determination. At least 50% viability needed for the assay. In the UDS assay, 1x10^5 cells/ml were seeded into coverslip cultures, exposed to ^{3}H-thymidine and test substance for 18.25 hours (3 cultures/dose level). Positive control was 2-acetyl aminofluorene (2-AAF, 0.2µg/ml). Cells growing on coverslips were rinsed, fixed and glued to microscope slides on day 2. On day 3, slides were dipped in autoradiographic emulsion and stored in the dark at 2-8^{0}C. Autoradiographs were developed, stained and coverslipped on day 17. Number of grains overlying 50 randomly selected nuclei/slide were counted. The highest of 3 cytoplasmic grain counts/cell were subtracted to obtain net nuclear grain count. Avg. net nuclear grain count/slide (sum of net nuclear grain count ÷ 50) and mean net nuclear grain count (avg. net nuclear grain count/slide ÷3) were calculated. Test slides with negative average net nuclear grain counts were scored as zero.
Results	
Genotoxic effects	Biphenyl feedstock induced toxicity in primary hepatocytes following 18.5hrs exposure beginning at 8µg/ml (84.3% relative viability) with continuing decreases in viability with increasing dose to the maximum dose of 2048µg/ml (15% relative viability). Toxicity in the UDS assay occurred in the 50 and 100µg/ml dose groups resulting in fewer than 150 viable cells available for counting in each of these groups. Despite this toxicity, a positive, dose-related response for UDS was obtained at all dose levels. Mean net nuclear grain counts were 0.77, 0.00, 15.12, 68.46, 78.42, 251.91, and 248.28 for untreated medium control, vehicle F68 control, 5, 20, 50, 100µg/ml biphenyl feedstock, and 2-AAF positive control, respectively.

Conclusions (contractor)	Biphenyl feedstock induced dose related unscheduled DNA synthesis in cultured rat hepatocytes at all doses evaluated. Biphenyl feedstock causes DNA damage and excision repair in this assay.
Data Quality *Reliabilities*	**1**. Reliable without restrictions. Study conforms to standard design. GLPs have been followed.
Reference	Brecher, S., Goode, J.W. 1984. Hepatocyte primary culture/DNA repair test of Biphenyl feedstock. Proj. #2078. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Williams, G.M. 1977. Cancer Res. 37: 1845-1851 Williams et al. 1977. In Vitro 13: 809-817 Williams et al. 1982. Mut. Res. 97:359-370
Other *Last changed*	6/10/2001 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vitro

Test Substance	Biphenyl Feedstock, CAS #68989-41-3. Amber-colored liquid with aromatic odor.
Test substance	Compositional analysis, purity and stability referred to sponsor.
Method	
Method/guideline followed	Standard method based on Cortesi et al (1983), Dunkel et al (1981), Reznikoff et al (1973)
Type	In vitro cell transformation
System of testing	Mouse embryo cells
GLP	Yes
Year	1983
Species/Strain	BALB/3T3-A31-1-1 from T. Kakunaga, National Cancer Inst., 1982
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Cytotoxicity: 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048µg/ml; Transformation: 4, 8, 16, 32µg/ml, all diluted in 10% Pluronic® polyol F68 (prepared in deionized water, mol. wt. 8350, 80% hydrophilic).
Exposure period	2 days
Statistical Methods	None employed. Criteria for positive response were a two-fold increase in type III foci at the highest dose over vehicle control (at least 2 type III foci if vehicle control had none) with or without a dose related response, or a two-fold increase at two or more consecutive doses. Test is equivocal if two-fold increase occurred at any one level other than the highest acceptable dose.
Remarks for Test Conditions	Sufficient Biphenyl feedstock was weighed separately for each dose level and melted in an 85^0C water bath; 0.78ml of 10% F68 added per ml of final volume and balanced salt solution was added as required to achieve final volume for testing. Test preparations were stored at 50-60^0C until just prior to dosing when the preparations were mixed and added at 50µl to each 5 ml culture. All cultures were incubated at 37^0C in 5% CO_2 enriched humidified atmosphere. For cytotoxicity, 2 flask cultures/dose group, 2 cultures for vehicle F68 or medium negative control were seeded with $1x10^4$ cells/culture in day 1, exposed on days 2-3, trypsinized and counted with a Coulter Model ZB on day 4 for at least 20% survival. For transformation, 15 flask cultures ($1x10^4$ cells/culture/dose group)) and two colony formation flask cultures (100 cells/culture/dose group) were seeded on day 1, exposed on days 2-3 and culture medium changed on day 4. For transformation cultures, medium continued to be changed weekly to day 29. Positive control was 3-methylcholanthrene (1µg/ml). Colony formation cultures were fixed, stained, and counted visually on day 8 to determine cloning efficiency (avg. number colonies/flask ÷ 100 cells seeded). Transformation cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci ÷ total flasks/dose group.
Results	
Genotoxic effects	Biphenyl feedstock induced toxicity in BALB/3T3 cells after two days exposure beginning at 16µg/ml (49% viability); inducing reduction to 20% viability between 32-64µg/ml and 2.1% viability at 2048µg/ml. In the transformation assay, a progressive increase in cytotoxicity occurred with increasing doses from 8-32µg/ml reducing the relative cloning efficiency (rel. C.E.) from 72.5-27.5%, respectively. At 32µg/ml, the toxic response of 27.5% rel. C.L.was comparable to that of the positive control, 3-methylcholanthrene (28.4% rel. C.E.). The positive control induced the expected response for transformation: 10 type III foci. The vehicle control had 1 type III focus, but the untreated medium control was slightly higher with 2 type III foci. The 8µg/ml and 32µg/ml biphenyl feedstock cultures each had 2 type III foci compared with one type III focus in the vehicle control. However, since the untreated control also had 2 type III foci, the results from treated cultures were considered negative.
Conclusions	Biphenyl feedstock did not induce significant transformation in BALB/3T3 cells under conditions of this assay.

(contractor)	conditions of this assay.
Data Quality *Reliabilities*	**1.** Reliable without restriction.
Reference	Brecher, S, and Go ode, J.W. 1983. BALB/3t3 transformation test: Biphenyl Feedstock. Proj. #2079. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co, Houston, TX Cortesi, E. et al. 1983. Teratogenesis, Carcinogenesis, Mutagenesis 3: 101-110. Dunkel, V.A. et al. 1981. J. Nat'l Cancer Inst. 67: 1303-1315. Reznikoff, C.A. et al. 1973. Cancer Res. 3239-3249.
Other *Last changed*	6/07/2001 (prepared by a contractor to the Olefins Panel).

Genetic Toxicity - in Vivo

Test Substance	Biphenyl Feedstock, CAS #68989-41-3. Amber-colored liquid with aromatic odor.
Remarks	Compositional analysis, purity and stability referred to sponsor.
Method	
Method/guideline followed	Comparable to standard assay
Type	Mammalian bone marrow erythrocyte micronucleus
GLP	Yes
Year	1984
Species	Mouse
Strain	Crl:CD®–1 (ICR) BR Swiss
Sex	Male and female: 10M, 10F/group; 15M, 15 F in 1 group
Route of administration	Oral gavage
Doses/concentration levels	0, 0.25, 0.5, 1.0g/kg in corn oil
Exposure period	1 dose/day for 2 days; 1 group- 1 dose, 1 day only
Statistical methods	Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant ($p<0.05$) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met.
Remarks for Test Conditions.	Biphenyl feedstock dosing solutions were prepared fresh for each day of dosing –2.5 g was mixed with corn oil to make 50 ml, blended by shaking. In an initial study at doses of 0.0, 0.75, 1.5, 2.0, and 3.0g/kg, very high mortality occurred at doses of 1.5-3.0g/kg and the study was terminated. A new study was initiated at doses of 0.25 –1.0g/kg in a single dose daily by gavage for 2 days All mice were weighed on day 1 and on day of sacrifice. One half of each treated group and vehicle control (5M, 5F) was killed on day 3 and the remainder on day 4. One group (15M, 15F), given 1.0 g/kg by gavage in a single dose for 1 day only, was killed on days 2, 3, 4 (5/sex/day). Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of femoral bone marrow smears were prepared, stained with May-Grunewald /Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all associated mature erythrocytes (NORMs) were counted. Data collected included group mean body weights for each day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN.
Results	No mortality occurred at any dose level and no effects on body weight were observed in either sex. Mice treated with biphenyl feedstock did not show any significant change in the frequency of micronucleus formation in PCE and no significant changes in the ratio of PCE/NORM compared to vehicle controls. Positive and negative controls performed appropriately.
Genotoxic effects	
NOAEL (NOEL)	NOEL(genetic and systemic) = 1.0g/kg
LOAEL (LOEL)	
Conclusions (study authors)	Oral treatment of mice with Biphenyl feedstock for 1-2 days at doses up to 1.0 g/kg/day had no effect on frequency of micronucleated polychromatic erythrocytes in bone marrow. Under these test conditions LPFO does not induce cytogenetic damage.
Data Quality *Reliabilities*	**1.** Reliable without restrictions. Study conforms to standard design. GLP have been followed.
References	Khan, S.H. 1984. Micronucleus test in mouse bone marrow: Biphenyl feedstock administered orally for 2 days. Proj. #2077. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other *Last changed*	6/07/2001 (Prepared by a consultant to the Olefins Panel)

Repeated Dose Toxicity

Test Substance	Biphenyl Feedstock, CAS #68989-41-3. Dark brown liquid with crystal aggregrates. Refer to sponsor for further information
Remarks	
Method	
Method/guideline followed	None specified. Comparable to standard study
Test type	Sub-acute
GLP	Yes
Year	1983
Species	Rat,
Strain	Fischer 344
Route of administration	Dermal
Duration of test	12 days
Doses/concentration levels	0, 1.0, 2.0g/kg (diluted in corn oil to constant volume of 4ml/kg for administration)
Sex	5M, 5F/dose group
Exposure period	6hr/day
Frequency of treatment	days 1-5, 8-11
Control group and treatment	5M, 5F; 4ml corn oil/kg
Post exposure observation period	None
Statistical methods	Mean and standard deviation, Bartlett's test, and analysis of variance. Dunnett's test if data were homogeneous; modified t-test if data were non-homogeneous.
Test Conditions	Rats were housed individually in suspended, stainless steel cages with mesh fronts and bottoms, equipped with an automatic watering system. Water and rat chow diet were available ad lib. Room temperature was maintained at 74^0F with a relative humidity of 55% and 12 hour light/dark cycle. Backs of rats were clipped of hair and dosed dermally on days 1-5 and days 8-11 at 0, 1.0, and 2.0g/kg diluted in corn oil. Elizabethan collars were applied for the 6 hour exposure period, after which collars were removed and skin wiped free of test article. Rats were sacrificed on day12. Rats were observed for mortality and moribundity twice daily on dosing days and once daily on non-dosing days. Body wt was recorded 5 days before initiation and on days 1, 8, and before sacrifice on day 12. Rats were observed once daily for clinical signs on dosing days. Dermal effects were scored twice on each dosing day - before application and after removal. Blood was taken for clinical chemistry and hematology 7 days prior to initiation and on day 12 prior to sacrifice; parameters were total leukocyte count, total erythrocyte count, total platelets, hemoglobin, hematocrit, mean cell vol., mean cell hemoglobin, mean corpuscular hemoglobin, BUN, creatinine, alkaline phosphatase, sodium, potassium, glucose, SGOT, SGPT, total protein, albumin, and albumin/globulin ratio. All animals received a gross necropsy and potential target organs/tissues (liver, brain, heart, spleen, kidneys, testes) were weighed and preserved for histopathology. Certain organs/tissues (lung, skin, ovaries) were examined microscopically but not weighed. Only control and high dose groups were scheduled for histopatholgy.
Results	LOEL = 1g/kg based on reduction in body wt. (Assigned by reviewer)
NOAEL (NOEL)	
LOAEL (LOEL)	
Remarks	No mortality or moribundity was observed during the study. Body wts of males and females in groups 2 and 3 were reduced but the effect was statistically significant only in females. No dermal reactions or test article related clinical signs were observed. There were no biologically significant test article related changes in the hematology or clinical chemistry parameters. However, sera of test article-treated rats were more yellow than that of controls. There was an increase in absolute wt. of kidneys of males and females in the test article groups that was significant only for female left kidney. There were no gross pathological changes in skin at the site of application. There were no histopathological lesions that could be attributable to test article exposure.
Conclusions	Dosing with the test article did not produce overt toxicological effects but there were decreases in terminal body wt and increases in specific organ wt that appeared to be treatment related
(study authors)	

Quality Reliabilities	**2**. Reliable with restrictions. Dosing solution concentrations were not verified by analysis.
References	Rausina, G.A. 1983. Two-week repeated dose toxicity study in rats using Biphenyl Feedstock. Proj. #2044. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other Last changed	6/07/2001 (Prepared by a contractor to the Olefins Panel)

Repeated Dose Toxicity

Test Substance	Biphenyl Feedstock, CAS #68989-41-3. Amber-colored, semi-solid with aromatic odor.
Remarks	Refer to sponsor for further information.
Method	
Method/guideline followed	None specified. Comparable to standard study
Test type	Sub-acute
GLP	Yes
Year	1983
Species	Rat
Strain	Fischer 344
Route of administration	Inhalation
Duration of test	12 days
Doses/concentration levels	0, 1.07, 3.04g/m^3
Sex	5M, 5F/dose group
Exposure period	6 hr/day
Frequency of treatment	days 1-5, 8-11
Control group and treatment	5M, 5F; filtered air
Post exposure observation period	None
Statistical methods	Analysis of variance, Dunnett's test, Kolmogorov-Smirnov two-tailed test
Test Conditions	Rats were housed individually in stainless steel screen-bottomed cages in a room maintained at 75^0F with 37% relative humidity and 12 hour light/dark cycle. Chow diet and water were provided ad lib except during exposure. Test substance was aerosolized with a ball jet nebulizer heated to 70^0C to decrease viscosity. Chamber concentrations were determined by fluorescence spectroscopy. Both vapor phase and particulate phase concentrations were determined. Actual total exposure (and % of total as particulate) were 0, 1.07g/m^3 (60%) and 3.04g/m^3 (51%). Particulate mass median aerodynamic diameter was 5.2-5.4 microns. Rats were exposed to test article or filtered air for 6hr/day on days 1-5 and days 8-11; they were observed twice daily on dosing and once on non-dosing days for mortality and moribundity, and immediately after dosing for clinical signs. Body wt was recorded immediately prior to exposure on days 1 and 5, and prior to sacrifice on day 12. Non-fasted blood was collected on day 12 for blood chemistry and hematology. Measured chemistry parameters were BUN, creatinine, total protein, albumin, albumin/globulin ratio, and SGPT. Hematology parameters were white blood cells, red blood cells (RBC), hemoglobin, hematocrit, mean RBC cell vol., mean RBC corpuscular vol., and mean corpuscular hemoglobin. All rats were necropsied for gross lesions. Organs weighed were liver, brain, heart, kidneys, spleen, testes, and lungs; these organs as well as ovary, uterus, eyes and nasal turbinates were saved for histopathological examination. Only organs/tissues from control and high dose rats were examined.
Results	
NOAEL (NOEL)	LOEL = 1.07g/m^3 based on body wt loss. Assigned by reviewer.
LOAEL (LOEL)	
Remarks	There were no test article-related deaths during the study. Males and females of groups 2 and 3 showed dose related weight loss. Most of the group 2 and 3 rats showed perianal soiling, excessive ocular porphyrin, dry red matter around mouth and nose, and crystalline test article on fur. There were no biologically significant effects of test article on clinical chemistry or hematology parameters. However, there was a dose related yellowing of blood sera. There were significant increases in absolute liver and kidney wt of high dose females, and decreases in spleen wt of high dose males. There were many significant increases in organ/body wt ratios owing to decreased wt gain in treated rats. There were no microscopic changes in male or female organs/tissues attributable to test article exposure. The only effect noted at gross necropsy was skin discoloration in test article treated rats.
Conclusions	
(study authors)	Repeated exposure to 1.07g/m^3 or 3.04g/m^3 of test article caused no mortality. Several exposure-related clinical effects were observed including perianal soiling, respiratory and ocular discharges, and decreased body wt. In addition, at the high dose, female liver and

Quality Reliabilities	kidney wts were increased and male spleen wt was decreased. **1.** Reliable without restrictions
References	Gordon, T. 1983. Nine-day repeated dose inhalation study in rats, Biphenyl Feedstock. Proj. #2045. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other Last changed	6/07/2001 (Prepared by a contractor to the Olefins Panel)

Invertebrate Acute Toxicity

Test Substance	Biphenyl feedstock, CAS #68989-41-3
Method	
Method/guideline followed	None specified. Comparable to standard methods
Year (guideline)	Unknonn
Type (test type)	Acute Daphnid Flow-through Toxicity Study
GLP	Yes
Year (study performed)	1984
Species	Water flea (*Daphnia magna*)
Analytical Monitoring	Yes (total organic carbon)
Exposure Period	48 hours
Statistical Methods	Probit procedure of SAS. Chi square on each dose-response curve to verify non-heterogeneity and goodness of fit.
Test Conditions Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, supplier of organisms, age, size, loading	First instar (24-hour old) offspring of 20 gravid Daphnia were used as test animals in a flow-through proportional diluter system. One day prior to introduction into the flow-through system, 1000 mg/L nominal concentration of biphenyl feedstock was prepared by mixing 14.4 g of test material into 14.4 L water. The stock solution was continuously stirred while being dispensed into the diluter. Test concentrations of 0, 10, 18, 32, 56, 100 mg/L (nominal) were produced by dilution with volumes of control water. Flow rate through the system was 14.4 L of test preparation dispensed every 24 hours (25 ml dispensed during each 2.5 min/cycle. The flow-through system delivered 100 ml test solution to each dose level every 2.5 min/cycle (50 ml into each 3.0 L capacity duplicate flask). Starting at one end of the bioassay table, 2 daphnids were placed into a floating polypropylene cylindrical container with a mesh bottom in each test vessel (2 vessels/dose) in consecutive order to the end of the table, procedure was repeated going in reverse direction, then back again until each vessel contained 10 daphnids. Use of the cylindrical container in the test vessel allowed water to pass through, improved observation of the animals and prevented daphnids from escaping and being accidentally discharged at the overflow standpipe. All acclimation and test vessels contained charcoal filtered municipal water, pH 7.8-8.5, at a temperature range of 18.4-20.3^{0}C and daily photoperiod of a continuous 12 hour light/dark cycle. Daily records were kept on number of immobilized and dead animals/vessel at 6, 24, and 48 hours. Water temperature was recorded daily. Dissolved oxygen conc. (9.3-10.1 mg/L), pH, total alkalinity (31 mg/L as $CaC0_3$) specific conductivity (297-311 umhas/om), and hardness (80-82 mg/L as $CaC0_3$) were measured during the first 6 hours, and at 48 hours in each vessel. Water samples for chemical analysis of actual concentration by fluorescence spectrometer (410 nm wavelength) were collected on day 0 and at 48 hours from each bioassay vessel. Actual concentration was calculated by subtracting the mean background TOC value of 6.8 mg/L from analytical test material concentrations at each dose level. The positive control compound, hexavalent chromium as potassium dichromate was detected in water samples by atomic absorption spectrometry.
Results	
Units/Value: Note: Deviations from protocol or guideline, analytical method, biological observations, control survival	EL_{50}(24-hour) = > 100 mg/L (95% Confidence limits not determined) based on nominal concentrations EL_{50}(48-hour) = 23.6 mg/L (95% Confidence limits = 17.5 - 30.7 mg/L) based on nominal concentrations Percent mortality at 48 hours was 0, 22, 39, 44, 89, 100% for untreated control (0), and nominal concentrations of 10, 18, 32, 56, 100 mg/L, respectively. Actual water soluble concentrations of biphenyl feedstock were 0.1, 1.7, 2.7, 3.8, and 7.5 mg/L at nominal concentrations of 10, 18, 32, 56, 100 mg/L, respectively. No treatment related changes in any water characteristic measurement were induced by test material or positive control. The 24-hour and 48-hour EC_{50} values for hexavalent chromium were 0.18 and 0.13 mg/L

	The 24-hour and 48-hour EC_{50} values for hexavalent chromium were 0.18 and 0.13 mg/L, respectively.
Conclusions (study author)	
Data Quality *Reliabilities*	**(1)** Reliable without restriction
Reference	Meyers, W.R., Rausina, G.A. 1984. 48-hour Aquatic Toxicity Study in *Daphnia* with Biphenyl Feedstock. Project #2042. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemical Co., Houston, TX, USA.
Other *Last changed*	American Chemistry Council, Olefins Panel

Biodegradation

Test Substance	Biphenyl Feedstock, CAS #68989-41-3
Method	
Method/guideline followed	OECD guideline 301D; EEC directive 67/548 Annex V part C.6 (84/449/EEC)
Year (guideline)	1984
Type (test type)	Aerobic Aquatic Biodegradation (Closed Bottle Test)
GLP	Yes
Year (study performed)	1993
Inoculum	Domestic activated sewage sludge bacteria from Huntingdon Research Centre sewage treatment plant
Exposure Period	28 days
Test Conditions Note: Concentration prep., vessel type, replication, test conditions.	Biphenyl feedstock was initially dissolved in chloroform to give a stock solution of 560 mg/ml; 10 ml aliquots were adsorbed on Whatman GFA glass filter paper and solvent evaporated to dryness. One piece of filter paper was placed in each test bottle prior to filling with inoculated medium. Test bottles included non-inoculated nutrient medium, inoculated nutrient medium, inoculated medium plus blank filter paper, 2 mg/L biphenyl feedstock adsorbed on filter paper, 3 mg/L sodium benzoate standard, and 2mg/L biphenyl feedstock adsorbed on filter paper +3 mg/L sodium benzoate to evaluate inhibitory effects. The nutrient medium consisted of aerated reverse osmosis purified, deionized water, phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride. Activated sewage sludge filtrate was added at a rate of 1 drop of inoculum/liter. Biochemical oxygen demand (BOD) dark glass 280 ml culture bottles, fitted with ground glass stoppers, were filled by siphon and firmly stoppered to exclude all air bubbles. Duplicate bottles were prepared in each test and control series to allow single oxygen determination/bottle at 0, 5, 15, and 28 days. The bottles containing biphenyl feedstock on filter paper +sodium benzoate were sampled on day 0 and 28. All bottles were incubated in a water bath at 20 ± 1^0C; measurements of dissolved oxygen conc. were made with a Yellow Springs BOD meter. Concentrations of biphenyl feedstock or sodium benzoate as mg carbon/L were not provided. Chemical oxygen demand (COD) was determined using a semi-micro sample digestion method. Test substance samples (50 µl) dissolved in chloroform were placed in clean, dry reaction vials and evaporated to dryness. Deionized water (2 ml), + sulfuric acid, potassium dichro mate, mercuric sulfate, and silver catalyst were added and reaction vial heated at 150^0C for 2 hours. COD values were read by spectrophotometer. Control blanks using solvent only and a sodium benzoate standard were also evaluated.
Results	
Units/Value: Note: Deviations from protocol or guideline, analytical method.	Percentage biodegradation was determined by comparing the oxygen depletion value with the corresponding Theoretical oxygen demand (NO_3)[ThOD($_{NO3}$)]. ThOD($_{NO3}$) was 2.94 mgO_2/mg for biphenyl feedstock (supplied by sponsor) and 1.67 mg O_2/mg for sodium benzoate. Chemical oxygen demand (calculated) for biphenyl feedstock was slightly lower at 2.38 mgO_2/mg; for sodium benzoate COD was 100% ThOD($_{NO3}$). Biphenyl feedstock attained 57% biodegradation within 28 days. Sodium benzoate degraded 86% within 28 days. Cultures containing both biphenyl feedstock and sodium benzoate showed an oxygen depletion value 4% higher than anticipated on the basis of separate cultures indicating that test material does not have an inhibitory effect on sewage bacteria. No inhibitory effects on activated sewage bacteria were observed in this assay.
Conclusions (study author)	
Data Quality *Reliabilities*	**(1)** Reliable without restriction
Reference	

Reference	Douglas, M.T. 1993. Biphenyl Feedstock Ready Biodegradability (Closed Bottle Test). CRTC Ref. #97-78. Huntingdon Research Centre Ltd. Cambridgeshire England for Chevron Research and Technology Co., Richmond, CA, USA.
Other *Last changed*	American Chemistry Council, Olefins Panel

Robust Summary – Group 10: Coal-Derived Fuel Oils

Acute Toxicity

Test Substance	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538^{0}C.
Method	
Method/guideline followed	None specified
Type (test type)	Acute limit test
GLP	Yes
Year	1984
Species/Strain	Rat/ Sprague-Dawley
Sex	Male and female
No.animals/sex/dose	5
Vehicle	None
Route of administration	Oral
Test Conditions	Animals were individually caged and maintained on a 12 hour light/dark cycle, at approximately 70^{0}F and 40% relative humidity. Food and water were provided ad lib. Rats were 6-12 weeks of age at study initiation. A single administration of 5 g/kg was given by gavage. Animals were observed for 14 days post-dose. Gross necropsy was conducted at termination.
Results	
LD_{50} with confidence limits.	$LD_{50} > 5$ g/kg.
Remarks	Three of the dosed rats died during the post-dosing period (Days 2, 3, and 6). Clinical signs observed in the surviving rats included ataxia, alopecia, abdominal griping, nasal discharge, urinary, fecal, and ano-genital staining, unthrifty coat, hypoactivity, wet and dry rales, and ocular discharge. The surviving rats all gained weight throughout the observation period. There were no significant findings at gross necropsy.
Conclusions (study author)	The test material was judged to be essentially non-toxic.
Data Quality	
Reliability	**1.** Reliable without restrictions.
References	McKee, R.H., Biles, R.W., Kapp, R.W. and Hinz, J.P. 1984. The acute toxicity of coal liquefaction-derived materials. J. Appl. Toxicol. 4: 198-205.
Other	
Last changed	14-Dec-01 (Prepared by a contractor to the Olefins Panel).

Acute Toxicity

Test Substance	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538^{0}C.
Method	
Method/guideline followed	NAS procedure, Draize scoring
Type (test type)	Acute dermal toxicity
GLP	Yes
Year	1984
Species/Strain	Rabbit/New Zealand White
Sex	Males and females
No. of animals/sex/dose	6
Vehicle	None
Route of administration	Dermal
Test Conditions	The study was designed to assess potential for systemic toxicity and skin irritation. Animals were individually caged and maintained on a 12 hour light/dark cycle, at approximately 70^{0}F and 40% relative humidity. Food and water were provided ad lib. Rabbits were about 12 weeks of age at study initiation. A single administration of 3.16 g/kg was applied to the clipped dorsal surface of the back of 6 rabbits (3 with abraded backs, 3 non-abraded), and covered with a gauze patch under a plastic sleeve. Patches were removed after 24 hours. Observations were made at 24 hrs post-dose and then daily for a total of 14 days; dermal responses were evaluated 30 min. after dosing, and at 2, 3, 7, 10 and 14 days post-dose. A gross necropsy was performed at terminal sacrifice.
Results	
LD_{50} with confidence limits.	LD_{50} > 3.16 g/kg.
Remarks	No animals died during the study. Clinical signs observed during the 14-day observation period included emaciation, nasal discharge, soft stool, and alopecia. Dermal observations included severe erythema and moderate edema; positive dermal scores persisted to study termination. Rabbits also exhibited atonia, fissuring, exfoliation, eschar and desquamation. Both males and females exhibited slight weight loss from study initiation to Day 14.
Conclusions (study author)	The test material produced significant dermal irritation and skin injury as evidenced by fissuring, desquamation, and eschar formation.
Data Quality Reliability	**1.** Reliable without restrictions
References	McKee, R.H., Biles, R.W., Kapp, R.W. and Hinz, J.P. 1984.. The acute toxicity of coal liquefaction-derived materials. J. Appl. Toxicol. 4: 198-205. National Academy of Sciences, 1977. Principles and procedures for evaluating the toxicity of household substances. Pp. 23-57. Washington, DC Draize, J.H. et al. 1944. Pharmacol. Exp. Therapeut. 82: 377 –390.
Other	
Last changed	14-Dec-01 (Prepared by a contractor to the Olefins Panel).

Genetic Toxicity - in Vitro

Test Substance	
Test substance	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538^{0}C.
Method	
Method/guideline followed	Standard method per Ames et al. 1975, 1983
Type	Reverse mutation bacterial assay
System of testing	Salmonella typhimurium with and without metabolic activation
GLP	Yes.
Year	1984 (1995 publication)
Species/Strain	Salmonella typhimurium TA100, TA98
Metabolic activation	Yes
Species and cell type	Sprague-Dawley rat or Syrian Golden hamster liver (S9 fraction), sex not specified
Quantity	50ul S9 fraction/plate (0.1ml S9 fraction/1.0ml S9 mix)
Induced or not induced	Aroclor 1254 induced; dosage and treatment not specified
Concentrations tested	0.0, 0.1, 1.0, 10.0, 50, 100, 500µg/ml ±S9, diluted in dimethyl sulfoxide (DMSO); additional doses with Tween 80 dispersant were 1000, 10,000µg/ml.
Statistical Methods	Mutation induction curve based on revertants/plate was plotted and mutagenic potency, the slope of revertants/µg dose of each induction curve was determined by non-linear regression analysis
Remarks for Test Conditions	Assays were conducted only in strains TA 100 and TA98 ±S9, the strains potentially most sensitive to detect activity by this type of comp lex hydrocarbon. Positive results were observed only in TA98+S9 and are the only data reported. Fresh bacterial stocks were exposed to graded doses of test material ±S9 from rat or hamster liver S9 (3 plate/dose/ liver fraction). Positive control was benzo(a)pyrene (5µg/plate); vehicle controls were DMSO or Tween 80. When detergent dispersant was utilized, equal volumes of test material and Tween 80 were mixed, distilled water was added dropwise to produce emulsions with final concentration of 10%(v/v) oil. Additional dilutions required for testing were made with 10% solutions of Tween 80 in distilled water to maintain constant detergent concentrations in test samples. Aliquots of these dilutions were plated with TA100 and TA98 ±S9 from rat or hamster liver. After 72 hr incubation at 37^{0}C, revertant colonies were counted with a Biotran III automatic colony counter.
Results	
Genotoxic effects	Fuel Oil blend produced a dose related increase in revertant frequency under all testing conditions in strain TA98+S9. Mutagenic potency was enhanced when hamster S9 was employed vs rat S9 (7.22 vs 5.29 revertants/µg, respectively). Detergent dispersion did not increase assay sensitivity for the fuel oil blend. Positive and negative controls performed appropriately; hamster S9 activation improved response of bacteria to benzo(a)pyrene but addition of dispersant did not enhance response beyond hamster S9 alone.
Conclusions	
(study authors)	EDS fuel oil blend was the most active bacterial mutagen of the high boiling coal liquids tested in this assay and contained the highest level of nitrogen. These positive results agree qualitatively with positive results in dermal carcinogenesis studies; however the lack of quantitative agreement may be related to the fact that the Salmonella assay is highly sensitive to aromatic amines and nitroaromatic compounds while dermal carcinogenesis is predominantly associated with neutral polycyclic aromatic hydrocarbons. Coal derived liquids containing substantial amounts of materials boiling above 370^{0}C are active in carcinogenesis screening assays.
Data Quality	
Reliability	**1.** Reliable without restrictions.
Reference	McKee, R.H., Traul, K.A., and Przygoda, R.T. 1995. Evaluation of coal liquids derived from EDS process in carcinogenesis screening tests. J. Appl. Toxicol. 15: 159-165 (see separate summary for cell transformation data) Ames, B.N. et al. 1975. Mutat. Res. 31: 347-364 Maron, D.M. and Ames, B.N. 1983. Mutat Res. 113: 173-215.
Other	
Last changed	14-Dec-01 (Prepared by a contractor to the Olefins Panel).

Genetic Toxicity - in Vitro

Test Substance	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538^{0}C.
Method	
Method/guideline followed	Standard method per Pienta et al, 1977; Przygoda et al, 1985
Type	Cell transformation
System of testing	Syrian Hamster embryo cells (SHE)
GLP	Not specified
Year	1984 (1995 publication).
Species/Strain	Cells isolated from eviscerated, decapitated day 13 embryos of timed-pregnant Syrian hamsters from Eagle Laboratories, Farmersburg, IN.
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	0.0, 0.1, 0.5, 1.0, 5.0, 10.0, 50, 100, 500, 1000µg/ml, diluted in dimethyl sulfoxide (DMSO)
Statistical Methods	None
Remarks for Test Conditions	Primary cultures of freshly derived hamster embryo cells were grown on Dulbecco's modified Eagles medium with 20% fetal bovine serum for 2 days, then frozen. Each assay consisted of concurrent tests in two distinct pools of embryo cells (each pool comprised of all cells from a single litter). Cell pools were screened with mutagenic and non-mutagenic compounds for validation prior to study initiation. Irradiated feeder cells were plated in 60 mm culture dishes; 24 hr later, non-irradiated target cells were plated at density of 300 cells/dish. One day later, test material was added to 3 plates/concentration/cell pool. Cells were grown for 7 days, then fixed and stained, and transformed colonies identified and counted. Total colony count and number of transformed colonies were recorded for each plate. Cloning efficiency and transforming efficiency were calculated. Assay was valid only if spontaneous and vehicle control cultures had no spontaneous transformed foci and the positive control, benzo(a)pyrene induced transformation at 2 doses (5, 10µg/ml).
Results	
Genotoxic effects	Morphological transformation in SHE cells was induced over a concentration range of 1-100µg/ml and was toxic to concentrations exceeding 100µg/ml. Results were comparable in both cell pools. Positive and negative controls performed appropriately.
Conclusions (study authors)	EDS experimental fuel oil blend induced transformation in SHE cells with approximately equal efficiency compared to two other coal derived high boiling liquids tested concurrently. Both neutral PAH and nitrogen-containing PAH fractions are active in this assay. The results of this assay agree with positive dermal carcinogenicity studies and demonstrate that coal derived liquids containing substantial amounts of materials boiling above 370^{0}C are active in carcinogenesis screening assays.
Data Quality	
Reliability	**1.** Reliable without restriction.
References	McKee, R.H., Traul, K.A., and Przygoda, R.T. 1995. Evaluation of coal liquids derived from EDS process in carcinogenesis screening tests. J. Appl. Toxicol. 15: 159-165. Pienta, J.A. et al. 1977. Int. J. Cancer 19: 642-655 Przygoda, R.T. et al. 1985. In Vitro 21: 32-38
Other	
Last changed	14-Dec-01 (Prepared by a contractor to the Olefins Panel).

Repeated Dose Toxicity

Test Substance	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538^{0}C.
Method	
Method/guideline followed	Other.
Test type	28-day dermal.
GLP	Yes
Year	1983 (1985 publication)
Species	Rabbit
Strain	New Zealand White
Route of administration	Dermal
Duration of test	4 weeks
Doses/concentration levels	0, 50, 200 mg/kg
Sex	Males and females (5/sex/dose)
Exposure period	4 weeks
Frequency of treatment	5 days/wk
Control group and treatment	5M, 5F; Primol 185 (highly purified mineral oil; white oil)
Post-exposure observation period	None
Statistical methods	Dunnett's test for comparing means of test groups and controls
Test Conditions	Rabbits (3-5kg) were individually housed in suspended steel cages in a room maintained between 18-22^{0}C, relative humidity of 40-70% and a 12 hr light/dark cycle. Food and water was available ad lib. Test material was applied to unabraded skin in an area of approx. 200cm^2 on the dorsal surface between the shoulders and lumber region. Elizabethan collars were used to minimize ingestion. Test material (50 and 200mg/kg) was diluted in Primol 185, and administered at 2ml/kg for 5 consecutive days/wk during the 4 wk dosing period. Residual test article was allowed to remain or accumulate on the skin. The 200mg/kg dose selected was the highest dose that did not cause weight loss in a 5 day range-finding study. Rabbits were clipped twice weekly during the 4 wk dosing period. Rabbits were observed daily for clinical signs of toxicity and skin irritation. Body weightt and food consumption were determined weekly. Blood was collected for hematology and clinical chemistry from unfasted rabbits prior to dosing, and at sacrifice (24 hr fasted). All animals were necropsied and organ wt determined for liver, kidney, epididymides and testes. Tissue preserved for histopathological examination included brain, heart, lungs, liver, kidney, spleen, testis, epididymis, prostate, seminal vesicle, ovary, urinary bladder, adrenal, pancreas, thymus, bone marrow, and skin.
Results	
NOAEL (NOEL)	NOAEL not determined
LOAEL (LOEL)	LOAEL = 50mg/kg based on body weight reduction, liver weight increase, serum cholesterol increase (estimated by reviewer).
Remarks	No mortality occurred during the study. At the site of application, desquamation, blanching, atonia and fissuring were observed in the high dose group. The low dose group and controls showed only a low incidence of desquamation. There was a dose-related decrease in mean group body weight of both males and females that became more pronounced over time; however, significance was reached only in the high dose females. Food consumption was also reduced but not to levels of statistical significance. All dosed rabbits showed statistically significant, dose-related increases in liver and kidney weight and organ to body weight ratios. Diffuse hepato-cytomegaly was evident in livers from 9/10 high dose and 4/10 low dose rabbits; effects ranged from slight to severe. Cytoplasmic degeneration and vacuolated hepatocytes were occasionally seen. Thymic atrophy was observed in 6/8 high dose rabbits but not in the low dose group. No other microscopic abnormalities were observed. Blood cholesterol levels were significantly elevated in a dose related manner in both sexes; other clinical chemistry values were within normal ranges.
Conclusions (study authors)	The test substance elicited dermal irritation as well as systemic effects that might have been related to weight loss or stress. Hepatic alterations were manifested as liver enlargement,

	elevated serum cholesterol, gross observations of liver abnormalities, and microscopic findings of diffuse hepatocytomegaly, cytoplasmic degeneration and hepatocellular vacuolization. Kidney weight and kidney weight to body weight ratio were elevated in both sexes, in a dose responsive manner, but there were no histopathological abnormalities observed.
Quality	
Reliabilities	**1.** Reliable without restrictions
References	McKee, R.H., Kapp, R.W., and Ward, D.P. 1985. Evaluation of the systemic toxicity of coal liquefaction-derived materials following repeated dermal exposure in the rabbit. J. Appl. Toxicol. 6: 345-351.
Other	
Last changed	14-Dec-01 (Prepared by a contractor to the Olefins Panel).

Repeated Dose Toxicity

Test Substance	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538^{0}C.
Method	
Method/guideline followed	Other.
Test type	90-Day oral toxcity.
GLP	Yes
Year	1984 (1987 publication)
Species	Rat
Strain	Sprague-Dawley (Hilltop Laboratory Animals, Scottdale, PA, 6 wks old at receipt)
Route of administration	Oral gavage.
Duration of test	Approx. 104 days
Doses/concentration levels	0, 0.02, 0.1, 0.5 g/kg/day diluted in highly refined white oil (CAS #8012-95-1)
Sex	Male and female (18M, 18F/dose group)
Exposure period	13 wks (90 days)
Frequency of treatment	Once a day, 5 days/wk
Control group and treatment	36M, 18F; 5ml/kg white oil; once a day, 5 days/wk
Post exposure observation period	2 weeks.
Statistical methods	Bartlett's test of homogeneity of variance, standard ANOVA, Duncan's test, linear regression for dose response; Kruskal Wallis (non-parametric test) followed by Dunn's Summed Rank test if appropriate; Jonckheere's test for monotonic trends in dose groups.
Test Conditions	This assay was the subchronic toxicity portion of a combined Reproductive/Subchronic toxicity study with an initial group size of 18M, 54F/dose group and 36M, 90F/control. Animals were assigned to 4 treatment groups based on body weight and were housed individually in stainless steel cages in rooms maintained at 20-24^{0}C temperature, 40-70% relative humidity and 12 hr light/dark cycle. Animals received water and food ad lib. Test material was administered by gavage 5 times weekly for 13 wks; body weight was recorded prior to dosage initiation (day 0) and weekly throughout the exposure period. At the end of 13 wks of treatment, 18 female rats/group were selected for the subchronic study and the others mated. All males were mated for 10 days. Fourteen days after termination of dosing and following confirmation of mating, blood samples were collected from males and 18 females /group. Animals were killed and necropsied. Brain, kidneys, adrenals and reproductive organs were weighed and eleven organs as well as gross lesions and tissue masses, if any, were collected and processed for histopathology. High dose and control groups were examined microscopically.
Results	
NOAEL (NOEL)	NOAEL= 0.1g/kg , based on elevated liver weight and hematology effects at 0.5 g/kg (assigned by reviewer).
Remarks	No treatment related mortality or significant differences in food consumption or clinical signs, except for urogenital staining in high dose animals were observed. Body weight gain was significantly reduced in high dose males by 7% in wks 6-7 of dosing; wt gain in high dose females and all other treated animals was similar to controls. No apparent abnormalities in gross examination of visceral organs and no effects on organ weight of males in any group were observed. Absolute liver wt was elevated and brain wt was reduced in high dose females ($p<0.05$); brain wt was not significantly different from controls as a fraction of body weight. No treatment related microscopic changes in tissue from either sex in the high dose group were observed compared to controls. There were no gross lesions or masses. Erythrocyte counts, hemoglobin and hematocrit were significantly reduced in high dose females ($p<0.01$); hemoglobin was reduced in high dose males. Dose related changes in serum cholesterol (elevated) and SGOT levels (decreased) in high dose animals but fell within normal historical range of biological values. Other clinical chemistry parameters were not significantly different from concurrent vehicle control values.

Conclusions (study authors)	Administration of EDS coal-derived experimental fuel oil at doses up to 0.5g/kg/day for 13 weeks induced slight systemic toxicity in male and female rats, including reduced body wt gain, elevated liver weight, reduced hematology values, and elevated cholesterol which are qualitatively consistent with results of repeat exposure studies of other coal-derived liquids of similar boiling range.
Quality Reliability	**2.** Reliable with restrictions. Analysis of dosing solutions was not performed for composition, purity or stability.
References	McKee, R.H., Plutnick, R.T. and Traul, K.A. 1987. Assessment of the potential reproductive and subchronic toxicity of EDS coal liquids in Sprague Dawley rats. Toxicology 46: 267-289 (See separate summary for reproductive study). McKee, R.H. et al J. Appl. Toxicol. 4: 198-205 (additional analytical data on fuel oil)
Other Last changed	14-Dec-01 (Prepared by a contractor to the Olefins Panel).

Toxicity to Reproduction

Test Substance	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538^{0}C.
Method	
Method/guideline followed	Other.
Test type	1-generation reproductive toxicity.
GLP	Yes.
Year	1985 (1987 publication).
Species	Rat.
Strain	Sprague-Dawley (Hilltop Laboratory Animals, Scottdale, PA, 6 wks old at receipt).
Route of administration	Oral gavage.
Duration of test	Approx. 142 days (90 days exposure, 10 days mating, 40-42 days gestation and lactation).
Concentration levels	0, 0.02, 0.1, 0.5 g/kg/day diluted in highly refined white oil (CAS #8012-95-1).
Sex	Male and female (18M, 36F/dose group).
Exposure period	13 weeks (90 days)
Frequency of treatment	once a day, 5 days/wk
Control group and treatment	36M, 72F; 5ml/kg white oil; once a day, 5 days/wk
Statistical methods	Bartlett's test of homogeneity of variance, standard ANOVA, Duncan's test, linear regression for dose response; Kruskal-Wallis (non-parametric test) followed by Dunn's Summed Rank test if appropriate; Jonckheeres test for monotonic trends in dose groups. Standard nested ANOVA for fetal body parameters by sex nested within dam and dam within dose group analysis followed by least significant differences technique.
Remarks for Test Conditions.	This assay was the reproductive portion of a combined Reproductive/Subchronic toxicity study with an initial group size of 18M, 54F/dose group and 36M, 90F/control. Animals were assigned to 4 treatment groups based on body weight and were housed individually in stainless steel cages in rooms maintained at 20-24^{0}C temperature, 40-70% relative humidity and 12 hr light/dark cycle. Animals received water and food ad lib. Test material was administered by gavage 5 times weekly for 13 wks; body weight was recorded prior to dosage initiation (day 0) and weekly throughout the exposure period. At the end of 13 weeks of treatment, 18 female rats/group were selected for the subchronic study and the others mated. All males were mated, then killed and necropsied for the subchronic study. 18M, 36F/dose group; 36M, 72F/control group were housed together (1M:2F) for 10 consecutive nights or until mating was confirmed by vaginal plug or sperm in a vaginal rinse = Gestation Day (GD) 0. Mated females were maintained untreated throughout gestation and lactation (LD) to postpartum day 21. Maternal body weight was recorded on GD 0, 7, 14, 21, and LD 0, 7, 14, 21 and all dams were examined for viability, toxicological effects and unusual behavior. Offspring were examined grossly and weighed at LD 0, 4, 14, 21. All dams and surviving litters were killed and grossly examined on LD21. Pups were necropsied and visceral organs and brain examined. Pups that died spontaneously were also necropsied unless precluded by autolysis or cannibalism.
Results	
NOAEL	NOAEL (maternal)= 0.5g/kg; NOAEL (offspring)=0.5g/kg (assigned by reviewer).
Remarks	No treatment related mortality or significant differences in body weight gain, food consumption or physical signs, except for urogenital staining in high dose animals were observed. Seven control animals, 1 low dose female, and 1 mid dose male were euthanized during the treatment period due to dosing injuries. Pregnancy indices were comparable in all dose groups: 80.9%, 86.1%, 88.9% and 83.3% in control, low, mid and high dose groups respectively. No significant differences were observed in length of gestation or in maternal weight gain during gestation. No treatment related effects were observed in mean litter size, live births or pup survival at LD 4, 14 and 21 or in pup body weight throughout lactation to weaning at LD21. Sex of pups was not reported. A low incidence of malformations occurred in all groups: Control: 4 pups in 4 litters – 2 tail abnormalities, 1 depressed

	sternum, 1 short snout. Low dose: 4 pups in 2 litters – 2 syndactyly, renal agenesis, no sex organs (1 agnatha), 1 syndactyly and renal agenesis, 1 pup with small kidney. Mid dose: 2 pups in 2 litters – tail abnormalities. High dose: 4 pups in 2 litters – 1 agnatha, 2 tail abnormalities, 1 misshapen skull. Syndactyly and renal agenesis have been reported as a spontaneously occurring genetic syndrome in Sprague-Dawley rats (Schreiner et al., 1979). Also, in a parallel study on another coal liquid, 2 pups with this syndrome were reported in 2 control litters.
Conclusions (study authors)	Coal-derived experimental fuel oil administered orally to male and female rats at doses up to 0.5g/kg for 13 weeks prior to mating did not adversely affect reproductive capacity or performance. There was no treatment-induced effect on overall incidence of abnormalities in any treated group or increased incidence of any specific class of malformations.
Data Quality *Reliability*	**2.** Reliable with restrictions. Analysis of dosing solution was not performed for composition, purity or stability.
References	McKee, R.H., Plutnick, R.T. and Traul, K.A. 1987. Assessment of the potential reproductive and subchronic toxicity of EDS coal liquids in Sprague Dawley rats. Toxicology 46: 267-289 (See separate summary for subchronic study). McKee, R.H. et al J. Appl. Toxicol. 4: 198-205 (additional analytical data on fuel oil) Schreiner, C.A. et al. 1979. Teratology 19: 46(A).
Other *Last changed*	14-Dec-01 (Prepared by a contractor to the Olefins Panel).

Developmental Toxicity

Test Substance	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538^{0}C.
Method	
Method/guideline followed	OECD 414
Type of Study	Developmental toxicity.
GLP	Yes.
Year	1984.
Species/Strain	Sprague-Dawley Rats.
Sex	Pregnant females.
Number/sex/dose	25 females/dose.
Route of administration	Oral gavage.
Exposure Period	Days 6-19 of gestation.
Concentrations	0, 0.1, 0.5, or 1.0 g/kg diluted in highly refined white oil at 5 ml/kg volume (CAS #8012-95-1).
Controls	Controls received 5 ml/kg of white oil.
Statistical methods	ANOVA, Bartlett's Test, Kruskal-Wallis non-parametric test, Fisher's Exact Test.
Remarks for Test Conditions	Physical examinations were performed and body weights were measured on gestational days 0, 6, 10, 15, and 20. Mated females were sacrificed on gestational day 20 and a gross necropsy was performed. Uteri and ovaries were weighed and corpora lutea were counted. The number of implantation sites, early and late resorptions, and live and dead fetuses were determined. Full term fetuses were examined for abnormalities, weight, and crown-rump distance. From each litter, the heads of half of the fetuses were preserved and examined, while the other half of the fetuses were examined for skeletal malformations and ossification variations.
Results	NOAEL maternal: 0.1 g/kg. NOAEL fetal: 0.1 g/kg.
Remarks	Maternal: Maternal body weight gain (GD 6-20) and uterine weight at term were significantly reduced in the mid and high dose groups. There also were increased clinical observations in these groups that included nasal, ocular, oral, and vaginal discharges, rales, and ungroomed appearance. Fetal: The mid and high dose groups exhibited significant increases in early embryonic resorptions with corresponding decreases in the mean number of live fetuses (86% and 25% compared to 100% in the control and low dose). The remaining fetuses in the mid and high dose group had significantly reduced fetal body weight and crown-rump distance. The overall number of fetal skeletal malformations were not significantly different from the controls, although the ratios of malformed fetuses per litter were significantly increased in the mid and high groups. Gross visceral abnormalities were observed only in the mid and high dose groups.
Conclusions (submitter)	Coal-derived experimental fuel oil administered orally to pregnant female rats was embryo-lethal and teratogenic in rats at doses that are maternally toxic. Under the conditions of this study, the test substance was not a selective developmental toxicant.
Data Quality Reliability	**2.** Reliable with restrictions. Analysis of dosing solution for composition, purity or stability. was not performed
Reference	Exxon Biomedical Sciences (1984) "Oral teratology study in rats," Unpublished study.
Date last changed	14-Dec-01 (Prepared by a contractor to the Olefins Panel).

Robust Summary - Group 10: Fuel Oils

Acute Toxicity

Test Substance	Light Pyrolysis Fuel Oil, CAS #68527-18-4. Water-white liquid. Refer to sponsor for purity and stability.
Method	
Method/guideline followed	Standard method (not referenced) with doses based on a previous limit test and two range-finding studies.
Type (test type)	Acute LD_{50}
GLP	Yes
Year	1984
Species/Strain	Rat, Fischer 344
Sex	Male and female
No. of animals per sex/dose	5
Vehicle	None
Route of administration	Oral
Test Conditions	Rats (74 days old, 113.4-201.2g) were individually housed in screen-bottomed cages in a room with 71.3^0F temperature, relative humidity of 46.4% and 12 hr light/dark cycle. Chow diet and water were available ad lib. Rats were fasted for 24 hrs prior to dosing at 2.50, 2.75, 3.00 and 3.25g/kg. Rats were observed at 1 and 4 hrs after oral gavage dosing on day 1, and at least once daily thereafter with exception of days 5, 6, 7, 13 and 14 (holidays and week-ends). Observations for mortality and moribundity were performed daily until sacrifice on day 15. Gross necropsies were performed on all rats. Oral LD_{50} were calculated by Probit analysis.
Results	
LC_{50} with confidence limits.	LD_{50} combined sexes (95% confidence limits) = 2.89g/kg (2.63-3.28g/kg). Females were somewhat more susceptible than males. No effects seen on body wt. The most frequently observed clinical signs were nasal and ocular discharges,
Remarks	lethargy, and soft stools. Gross necropsies revealed no adverse findings.
Conclusions	
(study author)	LD_{50} combined sexes (95% confidence limits) = 2.89g/kg (2.63-3.28g/kg).
Data Quality	
Reliability	**1.** Reliable without restrictions
References	Rausina, G.A., 1984. Acute oral toxicity study in rats of Light Pyrolysis Fuel Oil. Proj. #2101. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 10: Fuel Oils

Acute Toxicity

Test Substance	Light Pyrolysis Fuel Oil, CAS # 68527-18-4. Clear greenish-blue liquid. Refer to sponsor for purity and stability.
Method	
Method/guideline followed	Standard method (not referenced)
Type (test type)	Limit test
GLP	Yes
Year	1984
Species/Strain	Rat, Fischer 344
Sex	Male and female
No. of animals per sex/dose	5
Vehicle	None
Route of administration	Inhalation (whole body exposure)
Test Conditions	Rats (11 wks old, 100-172g) were individually housed in stainless steel, screen-bottomed cages in a room maintained at 72.9^{0}F, relative humidity of 51% and 12 hr light/dark cycle. Rats received chow diet and water ad lib, except during exposure. One group of 10 rats was exposed to aerosolized test article, generated by a ball jet nebulizer, for 4 hrs. A condensing flask was used to prevent large particles from entering the chamber. Actual chamber concentration was 4.95g/m^3 (range 3.89-5.89g/m^3) as determined by gas chromatography. Median aerodynamic diameter was 3.2 microns. Rats were observed for clinical signs immediately after exposure, at 1 and 4 hrs post-dose, and daily thereafter over 14 days. Observations for mortality and moribundity were made twice on weekdays and once on weekends. Body wt was determined prior to exposure on day 1, on day 8, and before sacrifice. Gross necropsies were performed on day 15.
Results	
LC_{50} with confidence limits.	LC_{50} was not reached at 4.95g/m^3. There were no deaths during the study, and no gross pathological changes were seen at necropsy. Immediately after exposure, all rats exhibited hair discoloration, perianal soiling, dry red material around nose/mouth, and rales. Incidences of rales and/or sneezing/wheezing were sporadically seen in both sexes until day 14. Nasal and ocular discharge, red nose, ocular porphyrin, and partial closing of the eyes were also evident during the post-exposure period. There was initia l body wt loss in both sexes by day8, with recovery to initial wt or above by day 15.
Remarks	
Conclusions (study author)	No deaths were observed during the study; therefore the LC_{50} was not reached at 4.95g/m^3. Major clinical signs included hair discoloration, nasal discharge, rales, and dry red material around nose/mouth
Data Quality	
Reliability	**1.** Reliable without restrictions
References	Rausina, G.A., 1984. Acute inhalation toxicity study in rats of Light Pyrolysis Fuel Oil. Proj. #2102. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vitro

Test Substance	Light Pyrolysis Fuel Oil, Gulf. CAS #68527-18-4. Water-white liquid. Composition
Test substance	analysis, purity and stability referred to sponsor.
Method	
Method/guideline followed	Standard method based on Hsie et al. (1981), O'Neill & Hsie (1979)
Type	In vitro mammalian cell forward mutation
System of testing	Chinese hamster ovary (CHO) cell culture
GLP	Yes
Year	1985
Species/Strain	CHO-K-1 heterozygous for hypoxanthine-guanine phosphoribosyl transferase (HGPRT+/-) from Oak Ridge National Laboratory, TN.
Metabolic activation	Yes
Species and cell type	Rat liver (S9) fraction purchased from Litron Labs, Rochester, NY
Quantity	1.0mg S9 fraction/ml treatment medium in range finding trial; 0.5mg S9 fraction/ml
Induced or not induced	medium in mutagenicity test
Concentrations tested	Aroclor 1254 induced (treatment not specified) Range finding (Cytotoxicity): 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 5000µg/ml ± S9; Mutagenicity test #1: (8, 16 cytotoxicity only), 25, 32, 50, 64, 128µg/ml ±S9; repeat +S9: 50, 64µg/ml, all diluted in 50% Pluronic® polyol F127 (prepared in absolute ethanol, mol. wt. 12,500).
Statistical Methods	Frequency of mutant colonies per million clonable cells, corrected for absolute survival by viability plates, was calculated and comparisons of treated cultures with vehicle controls made on transformed data using a two-tailed t-test (Irr & Snee, 1979) using the MUTANT computer program (Snee et al., 1981). Criteria for positive results were significant ($p<0.05$) increase in mutant colonies (HGPRT+/-? HGPRT-/-) at any dose level and a dose related response. If only one criterion is met, results are considered equivocal.
Remarks for Test Conditions	Sufficient Light Pyrolysis Fuel Oil (LPFO) was weighed separately for each dose level into 1ml volumetric flasks and stored overnight. The following day, 0.18ml of 50% F127 was added to each flask along with sufficient medium (Ham's F-12 without hypoxanthine) to achieve final 1ml volume for testing. All dosing preparations were vortexed just after addition of medium and just prior to use. All cultures were incubated at 37^0C in 5% CO_2 enriched, humidified atmosphere. Positive control mutagens were ethyl methanesulfonate (100µg/ml) for –S9 cultures, and benzo(a)pyrene (4µg/ml) for +S9 cultures. For range finding (cytotoxicity), each dose group was composed of 2 flasks, one –S9, one+S9, negative controls ± S9, seeded with $5x10^5$ cells on day 1. Cultures were exposed to test compound for 5 hours on day 2. On day 3, cells were trypsinized and counted with a Coulter Model ZB, then 200 cells were transferred into each of 3 60mm culture dishes. These viability plates were incubated until day 10, fixed in methanol and stained with Giemsa. Colonies were counted visually or with an Artek Model 981 colony counter. Absolute survival = total colony count ÷ number of cells seeded/flask. Relative survival = absolute survival in treated cultures ÷ vehicle control survival. Acceptable survival level is at least 10%. An S9 concentration test using both viability and mutagenicity plates was performed at doses of 16 or 50µg/ml LPFO and S9 concentrations of 0.25, 0.5, 1.0mg/ml to select optimal S9 concentration for the definitive mutagenicity test. For mutagenicity, cells were seeded on day 1 into 6 flasks/dose group, 3-S9, 3+S9; on day 2 approximately 10^6 cells were exposed to LPFO for 5 hours. Vehicle control had 12 flasks, 6-S9, 6+S9. On day 3, cultures with excessive cytotoxicity were discarded. From remaining cultures, 200 cells were seeded to each of 4 viability plates/dose level; incubated to day 10, fixed with methanol, stained with Giemsa, and colonies counted for survival. Expression cultures (10^5-10^6 cells/one dish/dose) were seeded on day 3; subcultured three times until day 10 when 200 cells were seeded on each of 4 viability plates/dose and $2x10^5$ cells seeded on each of 5 mutagenicity plates/dose with selective medium containing 10^{-5}M 6-thioguanine to allow expression of HGPRT mutation. Cultures were incubated undisturbed until day 17

	when they were fixed and stained. For mutagenicity, a ratio of total colony counts in mutagenicity plates over absolute survival in viability plates was calculated for each treatment group. Frequency of mutant colonies/million clonable cells was calculated and statistical comparisons with negative control data were made. A repeat mutagenicity test with metabolic activation at two doses was also performed
Results Genotoxic effects	In the range finding test, LPFO induced post treatment cell toxicity at all dose levels with and without S9 (cells $x10^5$/ml: -S9 5.3, 3.5, 3.7, 3.3 and 0.0 at 0[vehicle control], 8, 16, 32, and 64µg/ml; +S9 3.4, 3.0, 2.9, 1.1, 0.57 and 0.3 at 0[vehicle] 16, 32, 64, 128 and 256µg/ml). No reduction in colony counts were observed in any non-activated dose groups with sufficient cells to clone (8, 16, 32µg/ml); in S9 activated cultures, significant cloning toxicity occurred at 64 and 128µg/ml (Relative survival 4.1 and 19.5%). The S9 concentration selected for the definitive mutagenicity test was 0.5mg/ml based on the greatest number of mutant colonies observed at this concentration in cultures treated with 50µg/ml LPFO. In the mutagenicity test, cell toxicity occurred at LPFO doses of 32µg/ml and higher in –S9 cultures (cells$x10^5$/ml 5.0, 4.2, 2.4 and 0.67 at 0[vehicle], 32, 50 and 64µg/ml, respectively) and at 16µg/ml and higher doses in +S9 cultures (cells$x10^5$/ml 4.8, 4.2, 2.2, 2.3, 2.4 and 1.1 at 0[vehicle], 16, 32, 50, 64 and 128µg/ml, respectively. Cultures treated with 8 and 16µg/ml ± S9 were not subcultured for mutagenicity. Cloning efficiency decreased in +S9 cultures at all doses (Relative survival: 9.0, 2.5, 8.4 and 1.3% at 32, 50 64 and 128µg/ml, respectively). No significant increase in mutant colonies and no dose related response was observed in any culture - S9 treated with LPFO. Positive control compounds demonstrated appropriate responses (EMS-S9 118.1 mutants/10^6 clonable cells; B(a)P 198.1 mutants/10^6 clonable cells). Due to an unexpected high value in one outlier culture flask (340.2 mutants) in the 64µg/ml +S9 dose group, a repeat activated test was performed at does of 50 and 64µg/ml. No significant increases in number of mutant colonies or a dose response were observed in the repeat assay (24.8, 7.9 and 1.4 mutants/10^6 cells at 0[vehicle], 50 and 64µg/ml).
Conclusions (contractor)	Light Pyrolysis Fuel Oil did not induce a mutagenic response with or without metabolic activation in CHO/HGPRT cells at any dose level. Light Pyrolysis Fuel Oil does not cause gene point mutations under the conditions of this assay.
Data Quality *Reliabilities*	**1.** Reliable without restrictions.
Reference	Papciak, R.J. 1985. CHO/HGPRT test of Light Pyrolysis Fuel Oil. Proj. #2105. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Hsie, A.W. et al. 1981. Mut. Res. 86: 193-214 O'Neill, J.P. and Hsie, A.W. 1979. Banbury Report 2: 55-63 Irr, J.D. and Snee, R.D. 1979. Banbury Report 2: 263-275. Snee, R.D., Smith, R.L., and Irr, J.D. 1981. MUTANT. A computer program for the evaluation of short-term mutation test results. E.I. Dupont de Nemours Co.
Other *Last changed*	Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vitro

Test Substance	Light Pyrolysis Fuel Oil, Gulf. CAS #68527-18-4. water white liquid. Composition analysis, purity and stability referred to sponsor.
Test substance	
Method	
Method/guideline followed	Standard method based on Williams et al. (1977,1982)
Type	In vitro mammalian cell DNA repair assay
System of testing	Unscheduled DNA Synthesis (UDS) in primary hepatocyte cultures.
GLP	Yes
Year	1984
Species/Strain	Fischer 344 male rat (8 wks old)
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	8, 16, 32, 64, 128, 256, 512, 1024 µg/ml, diluted in 50% Pluronic® polyol F127 (prepared in absolute ethanol, mol. wt 12,000, 70% hydrophilic)
Exposure period	18 hours
Statistical Methods	None specified. Criteria for positive response are incorporation of radioactive precursor (^{3}H-thymidine) in cells that are not normally synthesizing DNA, indicating repair of damage. A positive response is defined as a mean net nuclear grain count at any treatment level that exceeds concurrent negative control by at least 6 grains/nucleus; negative control value must not exceed 5 grains. If this criterion is not met, a positive response can be identified if there is a significant difference ($p<0.01$) in % cells in repair at any dose level and negative control value. This indicator defines whether a small fraction of cells is undergoing repair (Casciano & Gaylor, 1983). A positive response need not be dose related.
Remarks for Test Conditions	Sufficient Light Pyrolysis Fuel Oil (LPFO) was weighed separately for each dose level, 0.46ml of 50% F127 added per ml of final volume and sufficient medium (Williams Medium E with 10% fetal bovine serum and insulin) added to achieve final volume. Test preparations were mixed just prior to addition at 30µl to each 3ml culture. The conc. of ^{3}H-thymidine (½ life 12.5 yrs.) used in these assays was 1mCi/ml. All cultures were incubated at 37^{0}C in 5% CO_2 enriched humidified atmosphere. No range finding assay was performed. In the UDS assay, $2x10^5$ cells/ml were seeded into coverslip cultures, exposed to ^{3}H-thymidine and test substance for 18 hours (3 cultures/dose level, 8 dose levels), untreated controls, vehicle F127control and positive control, 2-acetyl aminofluorene (0.01µg/ml). Cells growing on coverslips were rinsed, fixed and glued to microscope slides on day 2. On day 3, slides were dipped in autoradiographic emulsion and stored in the dark at 2-8^{0}C. Technical error required the emulsion step be repeated on day 15; first emulsion was peeled off and slides were dipped in fresh emulsion. Autoradiographs were developed, stained and coverslipped on day 22. Numbers of grains overlying 50 randomly selected nuclei/slide were counted. The highest of 3 cytoplasmic grain counts/cell were subtracted and this number was divided by a conversion factor of 2 to obtain net nuclear grain count. Avg. net nuclear grain count/slide (sum of net nuclear grain count ÷ 50) and mean net nuclear grain count (avg. net nuclear grain count/slide ÷3) were calculated. In addition, % cells in repair was determined for each dose level.
Results	
Genotoxic effects	LPFO induced significant toxicity in primary hepatocytes following 18 hours exposure, leaving too few cells for UDS analysis at doses = 64µg/ml. LPFO caused dose related UDS at all non-toxic levels. Percentage of cells in repair increased from the vehicle control value of 2.7% to 43.3% at 8µg/ml to 96.0% at 32µg/ml. Statistics were not applied because LPFO induced avg. net nuclear counts >6 grains above neg. controls at all non-toxic levels. Positive and negative controls gave expected responses.
Conclusions	
(contractor)	Light Pyrolysis Fuel Oil induced dose related unscheduled DNA synthesis in primary cultures of rat hepatocytes beginning at the lowest dose tested. Light Pyrolysis Fuel Oil

	cultures of rat hepatocytes beginning at the lowest dose tested. Light Pyrolysis Fuel Oil causes DNA damage and repair under conditions of this assay.
Data Quality *Reliabilities*	**2**. Reliable with restrictions. Limited specific data supplied. Statistical criteria described but statistical method not cited or employed
Reference	Brecher, S. 1984. Hepatocyte primary culture/DNA repair test of light pyrolysis fuel oil. Proj. #2107. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Williams, G.M. 1977. Cancer Res. 37: 1845-1851 Williams et al. 1977. In Vitro 13: 809-817 Williams et al. 1982. Mut. Res. 97:359-370 Casciano, D.A. and Gaylor, D.W. 1983. Mut. Res. 122:81-86
Other *Last changed*	4/11/2001 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vitro

Test Substance	Light Pyrolysis Fuel Oil, Gulf. CAS #68527-18-4. Water-white liquid. Composition
Test substance	analysis, purity and stability referred to sponsor.
Method	
Method/guideline followed	Standard method based on Cortesi et al (1983), Dunkel et al (1981), Reznikoff et al (1973)
Type	In vitro cell transformation
System of testing	Mouse embryo cells
GLP	Yes
Year	1984
Species/Strain	BALB/3T3-A31-1-1 from T. Kakunaga, National Cancer Inst., 1982
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Cytotoxicity: 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 5000µg/ml; Transformation: 20, 60, 90, 110µg/ml, all diluted in 50% Pluronic® polyol F127 (prepared in absolute ethanol, mol. wt. 12,000, 70% hydrophilic).
Exposure period	2 days
Statistical Methods	None employed. Criteria for positive response were a two-fold increase in type III foci at the highest dose over vehicle control (at least 2 type III foci if vehicle control had none) with or without a dose related response, or a two-fold increase at two or more consecutive doses. Test is equivocal if two-fold increase occurred at any one level other than the highest acceptable dose.
Remarks for Test Conditions	Sufficient Light Pyrolysis Fuel Oil (LPFO) was weighed separately for each dose level, 0.46ml of 50% F127 added per ml of final volume and medium(Eagles MEM with 10% heat-inactivated fetal calf serum) added as required to achieve final volume for testing. Test preparations were mixed just prior to addition to cultures at 50µl to each 5 ml culture. All cultures were incubated at 37^0C in 5% CO_2 enriched humidified atmosphere. For cytotoxicity, 2 cultures/dose group, 2 cultures for vehicle F127 or medium negative control were seeded with $1x10^4$ cells/culture in day 1, exposed on days 2-3, trypsinized and counted with a Coulter Model ZB on day 4 for at least 10% survival. For transformation, 15 cultures ($1x10^4$ cells/culture/dose group)) and two colony formation cultures (100 cells/culture/dose group) were seeded on day 1, exposed on days 2-3 and culture medium changed on day 4. For transformation cultures, medium continued to be changed weekly to day 29. Positive control was 3-methylcholanthrene (1µg/ml). Colony formation cultures were fixed, stained, and counted visually on day 9 to determine cloning efficiency (avg. number colonies/plate ÷ 100 cells seeded). Transformation cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci ÷ total flasks/dose group.
Results	
Genotoxic effects	LPFO induced toxicity in BALB/3T3 cells after two days exposure beginning at 32µg/ml (74.8% relative survival); viability dropped sharply at 128µg/ml (6% relative survival) and was 100% toxic at higher concentrations. LPFO did not induce transformed foci in excess of negative control cultures at any dose level. Toxicity was evident at 60µg/ml (10% relative cloning efficiency). Positive and negative controls gave expected responses.
Conclusions	
(contractor)	Light Pyrolysis Fuel Oil did not induce transformation in BALB/3T3 cells at any dose level under conditions of this assay.
Data Quality	
Reliabilities	**1**. Reliable without restriction.
Reference	Brecher, S. 1984. Cell transformation test of Light Pyrolysis Fuel Oil. Proj. #2108. Gulf

	Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co, Houston, TX Cortesi, E. et al. 1983. Teratogenesis, Carcinogenesis, Mutagenesis 3: 101-110. Dunkel, V.A. et al. 1981. J. Nat'l Cancer Inst. 67: 1303-1315. Reznikoff, C.A. et al. 1973. Cancer Res. 3239-3249.
Other *Last changed*	7/27/2001 (prepared by a contractor to the Olefins Panel).

Genetic Toxicity - in Vivo

Test Substance	Light Pyrolysis fuel oil, Gulf CAS #68527-18-4 Water white liquid. Compositional analysis, purity and stability referred to sponsor.
Remarks	
Method	
Method/guideline followed	Comparable to standard assay
Type	Mammalian bone marrow erythrocyte micronucleus
GLP	Yes
Year	1984
Species	Mouse
Strain	Crl:CD®–1 (ICR) BR Swiss
Sex	Male and female: Range finding (RF) 2M, 2F/group; Micronucleus 10M, 10F/group; 15M, 15 F in 1 group (11-12wks old at study initiation).
Route of administration	Oral gavage
Doses/concentration levels	RF:0, 1.25, 2.5, 5.0 g/kg in corn oil; Micronucleus: 0, 0.25, 0.5, 1.0 g/kg
Exposure period	1 dose/day for 2 days; 1 group- 1 dose, 1 day only
Statistical methods	Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant ($p<0.05$) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met.
Remarks for Test Conditions.	Light pyrolysis fuel oil (LPFO) dosing solutions were prepared fresh for each day of dosing – for RF 2.5 g LPFO mixed with corn oil to make 10 ml; for micronucleus 2.5 g LPFO mixed with corn oil to make 50 ml, blended by shaking. Based on results of range finding study, three groups of mice were given LPFO by oral gavage daily for two days. All mice were weighed on day 1 and on day of sacrifice. One half of each treated group and vehicle control (5M, 5F) was killed on day 3 and the remainder on day 4. One group (15M, 15F), given 1.0 g/kg by gavage in a single dose for 1 day only, was killed on days 2, 3, 4 (5/sex/day). Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of femoral bone marrow smears were prepared, stained with May-Grunewald /Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all associated mature erythrocytes (NORMs) were counted. Data collected included group mean body weights for each day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN.
Results	In the range finding study, all mice given LPFO at 5.0 or 2.5 g/kg died by day 3. At 1.25 g/kg, ½ females died, surviving mouse was lethargic, males appeared normal. In micronucleus test, 1/10 males given 1.0 g/kg (2 doses) died by day 3. No other mortality or significant weight changes were observed. Surviving mice treated with LPFO did not show any significant changes in micronucleus formation in PCE and no significant changes in the ratio of PCE/NORM compared to vehicle controls..
Genotoxic effects	
NOAEL (NOEL)	NOEL (genetic)= 1.0 g/kg. NOEL (systemic)= 0.5g/kg
LOAEL (LOEL)	
Conclusions	Oral treatment of mice with Light Pyrolysis Fuel Oil for 1-2 days at doses up to 1.0 g/kg/day had no effect on frequency of micronucleated polychromatic erythrocytes in bone marrow. Under these test conditions LPFO does not induce cytogenetic damage.
(study authors)	
Data Quality	**1**. Reliable without restrictions.
Reliabilities	
References	Khan, S.H. 1984. Micronucleus test of Light Pyrolysis Fuel Oil. Proj. #2106. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	7/27/2001 (Prepared by a consultant to the Olefins Panel)

Repeated Dose Toxicity

Test Substance	Light Pyrolysis Fuel Oil, CAS# 68527-18-4 Water white liquid
Remarks	
Method	
Method/guideline followed	No guidelines specified, comparable to standard study.
Test type	Subacute
GLP	Yes
Year	1985
Species	Rat
Strain	F344
Route of administration	Dermal
Duration of test	8 days
Doses/concentration levels	0, 1.0, 2.0 g/kg
Sex	Males and females
Exposure period	6 hr/day
Frequency of treatment	once/day for 5 days
Control group and treatment	Light paraffinic oil (2.16 ml/kg)
Post exposure observation period	2 days
Statistical methods	Analysis of Variance, Dunnett's test
Test Conditions	Rats were housed in suspended stainless cages with wire mesh bottoms and fronts with automatic watering in a room maintained at 72.8^0F, relative humidity of 56% and 12 hour light/dark cycle. Chow diet and water were provided ad lib. Doses were administered over 10% of body surface to the backs of rats clipped free of hair and fitted with Elizabethan collars to reduce ingestion. After 6 hours, collars were removed and residual oil wiped off. The test substance mixture was analyzed at initiation and termination of dosing period for concentration/stability/uniformity by fluorescence. Observations for mortality and moribundity were made daily, and for clinical signs daily on dosing days. Body weight was recorded at initiation and termination. Necropsy and gross pathology were conducted but histopathology, clinical pathology and organ weights were not obtained.
Results	
NOAEL (NOEL)	NOEL not determined.
LOAEL (LOEL)	LOEL = 1g/kg/day (based on skin irritation).
Remarks	No mortality or morbidity was observed and there were no test article related clinical signs. By day 5, males and females in both dose groups had well defined erythema, with some resolution and eschar formation by day 8. Slight edema was seen in the high dose males and females that resolved by day 8. A significant decrease in body weight was noted in high dose males.
Conclusions	No mortality or morbidity were observed. Group mean body weight was decreased in males in the 2.0 g/kg group. Skin irritation occurred in rats receiving 1.0 and 2.0 g/kg of test article that partially resolved after the 2 day recovery period with severe erythema/eschar formation in 4 rats (3M, 1F) in the high dose group.
Quality	**2**. Reliable with restrictions. This study was limited in scope, being restricted to
Reliabilities	determination of morbidity/mortality, clinical signs including scoring for skin irritation, body weight changes and gross necropsy.
References	Rausina, G. 1985. Five-day repeated dose dermal toxicity study in rats of light pyrolysis fuel oil. Proj. #2109A. Gulf Life Sciences Center, Pittsburgh, PA. For Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	2/15/2001 (Prepared by a contractor to the Olefins Panel)

Repeated Dose Toxicity

Test Substance	Light Pyrolysis Fuel Oil, CAS #68527-18-4. No analysis of purity or composition reported,
Remarks	referred to sponsor.
Method	
Method/guideline followed	No guidelines specified, comparable to standard study.
Test type	Subacute
GLP	Yes
Year	1985
Species	Rat
Strain	Fischer 344
Route of administration	Whole body Inhalation
Duration of test	4 weeks
Doses/concentration levels	0, 0.51, 1.26, 2.54 g/m^3 (actual)
Sex	Males and females (10/sex/group)
Exposure period	6 hr./ day
Frequency of treatment	5 days/wk. For 4 wks.
Control group and treatment	Filtered air, 6 hrs/day, 5 days/wk.
Post exposure observation period	None
Statistical methods	Analysis of variance, Dunnett's test, Kolmogorov-Smirnov test
Test Conditions	Rats were housed individually in stainless steel, screen-bottomed cages with automatic watering systems in rooms maintained at 72.7^0F with 59% relative humidity and a 12 hour light/dark cycle. Chow diet and water were provided ad lib except during exposure. Chamber concentrations were monitored by GC; peak areas were compared with those of liquid test article standards. Target dose levels were 0.5, 1.25, and 2.5 g/m^3 administered as an aerosol. (Actual levels cited above). Each rat was observed twice daily on weekdays and once daily on weekends for morbidity and mortality, and once daily (immediately after exposure) for clinical signs. Body weight was taken at initiation and weekly thereafter. Blood was taken from non-fasted rats via the orbital sinus. At study termination, rats were necropsied for gross lesions and organs/tissues (34/rat) were preserved for histopathological evaluation (0, 1.26 and 2.54 g/m^3 groups).
Results	
NOAEL (NOEL)	Not determined
LOAEL (LOEL)	LOEL = 0.51 g/m^3 (decreased. body wt, dec. hematocrit, hemoglobin and blood glucose)
Remarks	After 4 exposures to 2.5 g/m^3, 75% of the rats were found dead or moribund; remaining rats were sacrificed and only histopathological evaluations were performed on these rats. After the last exposure, 2 females at the 1.25 g/mg^3 were sacrificed moribund. No other mortality was observed. The most frequent clinical signs in all dose groups were ocular porphyrin and discharge, closing of the eyes and nasal discharge; the highest incidence was in the 2.5 g/m^3 group. Significant decreases in body weight were present in all test article exposed groups, and, with the exception of the spleen, were associated with consistent increases in organ to body wt. ratios. Both clinical signs and body weight changes were correlated with dose. Total white blood cell and platelet counts were elevated at 1.26 g/m^3, and occasionally at 0.51 g/m^3; decreased mean corpuscular hemoglobin was seen in females of both these dose groups, and in males at 0.51 g/m^3. White cell increases were attributed to increased numbers of segmented neutrophiles. Blood glucose levels were increased in males and females at 2.54 g/m^3 and in males at 1.26 g/m^3. Gross pathological effects included alopecia, perianal soiling, abnormal coloration of liver, lack of body fat. There was a variety of histopathological findings with the most severe being necrosis of cortical thymus lymphocytes especially in the high dose females. Atrophy of the thymic cortex was seen in 1.26 and 2.54 g/m^3 males and females. Changes in the thymus were accompanied by atrophy of splenic lymphoid tissue, especially in high dose females, lymphoid hyperplasia in lungs, and hypoplasia of bone marrow. Hyaline droplets were observed in male kidneys in the 1.26 g/m^3 and 2.54 g/m^3 groups. Generalized vascular congestion was seen in both

	sexes in the 1.26 g/m^3 and 2.54 g/m^3 groups which was particularly prominent in bone marrow, kidney, adrenal, lung and thymus.
Conclusions (study authors)	No mortality or moribundity was seen in the 0.54 g/m^3 group. Significant decreases were noted in mean body weight of both sexes in all groups receiving test material. Treatment was associated with significant histopathological effects including severe atrophy of the thymic cortex, hypoplasia of bone marrow and hyaline droplet degeneration of male kidney tubular epithelium. These changes were related to test material dose.
Quality Reliabilities	**1.** Reliable without restrictions.
References	Rausina, G. 1985. Four-week repeated dose inhalation toxicity study in rats of Light Pyrolysis Fuel Oil. Proj. #84-2111. Gulf Life Sciences Center, Pittsburgh, PA. For Gulf Oil Chemicals Co., Houston, TX
Other Last changed	2/20/2001 (Prepared by a contractor to the Olefins Panel)

Repeated Dose Toxicity

Test Substance	Pyrolysis Fuel Oil, (water and oil quenched). Tested as supplied by producer. Produced by pyrolysis at temperatures up to 950^0C, contained 300-500 ppm benzo(a)pyrene
Remarks	
Method	
Method/guideline followed	No guidelines specified
Test type	Chronic/cancer study – skin painting
GLP	No
Year	1977
Species	Mouse (40/group/dose)
Strain	C3H/HeJ
Route of administration	Dermal
Duration of test	Lifetime (28 mon)
Doses/concentration levels	One brushfull – Mon, Wed., Fri.
Sex	Not specified
Exposure period	Continuous, no test article removal
Frequency of treatment	3 times/wk.
Control group and treatment	Water- 1 brushfull 3 times/wk
Post exposure observation period	None
Statistical methods	Least squares to determine median latent period
Test Conditions	Doses of neat test article or water were brushed on to the backs of mice, clipped free of hair, three times/wk. Doses were applied consistently to the midline but qualitatively, with each dose described as a brushfull. At monthly intervals, papilloma or carcinoma indexes were calculated (100 times the ratio of number of mice with skin tumors divided by the effective group). Effective group was calculated as original number of mice at start divided by the number dead without tumors, but maintained constant after appearance of the median tumor. Median papilloma or carcinoma latent periods were also determined. Lesions were verified by histopathology.
Results	
NOAEL (NOEL)	Not applicable
LOAEL (LOEL)	Not applicable
Remarks	Results of the water-quenched and oil-quenched oils were essentially identical. Both samples were highly carcinogenic. For the water quenched oil, the papilloma and carcinoma indexes were 100 and 97.2, respectively, and the median papilloma and cacinoma latent periods were 10.2 and 12.2 months, respectively. For the oil quenched oils, the indexes were 94.4 and 94.4, respectively, and the latent periods 10.3 and 12.1 months. The malignant tumors were squamous call carcinomas.
Conclusions	
(study authors)	Pyrolysis fuel oil (both water and oil quenched) was carcinogenic in the mouse skin painting bioassay.
Quality	
Reliabilities	Reliable with restrictions. Although the method used the early qualitative procedure of actual skin painting rather than exact volume application, and the material was not analyzed, the results are unambiguous. The described process conditions and high B(a)P levels are consistent with a dermal carcinogenic response and the latency period was short enough to anticipate possible metastatic spread (Reviewer's comments).
References	Weil, C.S., Condia, N.I. 1977. Experimental carcinogenesis of pyrolysis fuel oil. Am. Ind. Hyg. Assoc. J. 38: 730-733.
Other	
Last changed	4/11/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Acute Toxicity

Test Substance	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+
Method	
Method/guideline followed	None specified, comparable to standard studies
Type (test type)	Acute
GLP	Not specified
Year	1977
Species/Strain	Rat, Wistar
Sex	Males and females
No. of animals per sex per dose	5
Vehicle	None
Route of administration	Oral gavage
Test Conditions	Male and five female Wistar rats (200-300g) were fasted for 18 hr prior to test article administration by the oral route. Test material was given by intubation at 6.67, 10.0, 15.0 and 22.5g/kg. Following intubation, rats were returned to their holding cages. Food and water were available ad lib. Rats were observed for signs of toxicity and pharmacological effects for 14 days following treatment. Data for the 15.0g/kg dose was taken from a prior project (#MB77-1855).
Results	
LD_{50} with confidence limits.	LD_{50} combined sexes = 14.5 (11.5-18.3)g/kg.
Remarks	Clinical signs were produced at all doses, but the time after dosing when symptoms were seen, was not reported. Effects at the 4 dose doses were: 6.67g/kg, diarrhea, piloerection, lethargy, ptosis; 10.0g/kg, lethargy, ataxia, tremors, coma, flaccid tone, piloerection; 15.0g/kg, lethargy, coma; and 22.5g/kg, coma, death. Rats died at 15.0g/kg (2M, 4F) and 22.5g/kg (5M, 5F).
Conclusions (study author)	LD_{50} combined sexes = 14.5 (11.5-18.3)g/kg. Toxicological effects were observed at all doses, but death did not occur at doses below 15.0g/kg.
Data Quality Reliability	**2.** Reliable with restrictions. Observation times, when symptoms appeared, were not reported.
References	Moreno, O.M., 1977. Report on oral LD_{50} in rats. Project #MB77-2027, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp, Paulsboro, NJ Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ Moreno, O.M., 1977. Report on a single dose oral toxicity study in rats. Project #MB77-1855, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp, Paulsboro, NJ
Other	
Last changed	12/18/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Acute Toxicity

Test Substance	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+
Method	
Method/guideline followed	None specified, comparable to standard studies
Type (test type)	Limit test
GLP	Not specified
Year	1977
Species/Strain	Rat, Wistar
Sex	Males and females
No. of animals per sex per dose	5
Vehicle	None
Route of administration	Oral gavage
Test Conditions	Five male and five female Wistar rats (200-300g) were fasted for 18 hr prior to test article administration (neat) by the oral route. Test material was given by intubation at 15g/kg. Following intubation, rats were returned to their holding cages and observed for signs of toxicity and pharmacological effects for 14 days.
Results	
LD_{50} with confidence limits.	LD_{50} combined sexes ~ 15g/kg (Estimated by reviewer)
Remarks	Two of 5 males died on days 1 and 5 (one each day) and 4 of 5 females died on day 2 (2 rats), day 3 (1 rat); day 5 (1 rat). Lethargy and coma were observed shortly after dosing. Higher mortality was seen in females
Conclusions (study author)	The combined-sex LD_{50} was approximately that of the 15mg/kg limit dose administered, with higher mortality seen in females. This dose is very high and approximates the maximum dose that could be placed in the rat stomach without causing aspiration. (Reviewer's comment)
Data Quality	
Reliability	**2.** Reliable with restrictions. Very little data was provided in the report related to housing conditions and time course of clinical symptoms. GLP adherence not reported.
References	Moreno, O.M., 1977. Report on a single dose oral toxicity study in rats. Project #MB77-1855, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp, Paulsboro, NJ Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ
Other	
Last changed	12/18/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Acute Toxicity

Test Substance	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+.
Method	
Method/guideline followed	None specified, comparable to standard studies
Type (test type)	Acute
GLP	Yes
Year	1980
Species/Strain	Rat, Sprague Dawley
Sex	Males and females
No. of animals per sex per dose	5
Vehicle	Air
Route of administration	Whole body Inhalation
Test Conditions	Five groups of Sprague Dawley rats were exposed to an aerosol of test article for a single 4 hr period at doses of 3.6±0.39, 5.2±0.08, 5.7±0.17, 8.9±0.88 and 9.3±0.79 g/m^3±SD. The aerosol had an equivalent aerodynamic diameter of 2.7µm±1.9 geometric SD. Rats were observed during exposure and daily thereafter for clinical signs over 14 days. Body wt was determined at initiation and at 7 and 14 days post-exposure. All rats were necropsied for gross abnormalities.
Results	
LC_{50} with confidence limits.	LC_{50} combined sexes ~ 6.0g/m^3
Remarks	During the exposure, dyspnea, salivation, nasal discharge, and prostration were observed. Dyspnea was observed in practically all rats at all concentrations. Salivation occurred in 8 or more animals exposed to 8.9g/m^3 or greater and to a lesser degree in animals exposed to 5.7g/m^3. Nasal discharge was observed in all groups to varying degrees. Prostration occurred in 6 or more rats given 5.7g/m^3 or greater and to a lesser degree in animals exposed to 5.2g/m^3 or less; prostration appeared to be concentration related. Males in groups exposed to 3.6 - 8.0g/m^3 weighed slightly more than pre-exposure wt at 7 days post-exposure; by 14 days post-exposure, body weight of these males was approx. normal. Females in groups given 3.6 and 5.2g/m^3 gained body wt at approx. a normal rate through 14 days post-exposure period. In the 5.7g/m^3 group females were below pre-exposure levels at 7 days post-exposure; by 14 days post-exposure, these females were at approx. normal body wt. The females in the 8.9g/m^3 group and both males and females in the 9.3g/m^3 group did not provide sufficient numbers of surviving animals to evaluate effects on body weight gain. At necropsy, a number of macroscopic abnormalities were observed but only those involving lung, liver and stomach were considered remarkable (specifics not provided).
Conclusions	
(study author)	LC_{50} combined sexes ~ 6.0g/m^3
Data Quality	
Reliability	**2.** Reliable with restrictions. The short report provided for review did not describe housing conditions, method of aerosol generation, and specifics on gross necropsy observations.
References	LC_{50} Acute inhalation toxicity evaluation in rats. 1980. IRDC, Mattawan, MI, for Mobil Chemical Co., Beaumont, TX
Other	
Last changed	12/18/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Acute Toxicity

Test Substance	140^0F vapor of Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Vapors were 30-35% alkanes/alkenes, 60-65% benzene derivatives, and 2-3% naphthalenes. Benzene, toluene, cyclopentadiene and styrene were 53% of the vapor.
Method	
Method/guideline followed	None specified, comparable to standard studies
Type (test type)	Acute
GLP	Yes
Year	1983
Species/Strain	Rat, Sprague Dawley
Sex	Males and females
No. of animals per sex per dose	10
Vehicle	Air
Route of administration	Whole body Inhalation
Test Conditions	Male and female rats (13wk old; males 349-368g, females 213-215g) were housed 5/sex/cage, in polypropylene cages with removable mesh tops and floors. Chow diet and water were available ad lib. Holding room temperature was 22 ± 0.8^0C, relative humidity was 63±3.0%, and a 12hr light-dark cycle was maintained. Vapors were continuously generated from a supply of neat oil at 140^0F in a double-surface glass condenser. Nitrogen gas carrier was passed through the condenser, diluted with air, and metered into the exposure chambers. Chamber vapor concentrations were monitored by an infrared gas analyzer, and samples were collected every 30 min for analysis by gas chromatography/ flame ionization detection. Rats received a single 4hr exposure to vapor (g/m^3 ±% variation) at analyzed concentrations of 0.59±21, 3.3±21, or 6.6±21. During exposure, rats were held in stainless steel mesh cages placed on supports in the inhalation chamber. After exposure, rats were returned to holding cages; 5 rats/sex/group were retained for a 24hr observation period, remaining 5 rats/sex/group were retained for a 14 day observation period. Rats were observed for clinical signs at 15 min intervals during exposure, 1 and 2 hrs after exposure, and once daily thereafter until sacrifice. Body wt. was recorded at initiation (day 0), on day 1, and at sacrifice; rats selected for 2wk observation were also weighed on days 2, 4, 7, and 14. Necropsies were performed on all rats. For the 24hr post-exposure sacrifice, liver, head, lungs and kidneys from all rats were weighed and processed for microscopic examination. For the 2 wk post-exposure sacrifice, lungs were weighed and processed for microscopic examination. Tissues collected from only the high-dose and control rats were examined. Final and initial body wt and organ wt were analyzed by covariance or analysis of variance, as appropriate with application of Bartlett's test.
Results	
LC_{50} with confidence limits.	LC_{50} was not reached at $6.6g/m^3$
Remarks	During exposure, visible rats showed partial eye closing and prone body posture in the 3.3 and $6.6g/m^3$ groups. Slight lacrimation and abnormal breathing was noted in most rats at the high dose. During the 14-day observation period, appearance and behavior were considered normal. There were no statistically significant changes in body wt. At the 24hr post-exposure necropsy, no gross abnormalities were observed. There were no biologically significant changes in organ wt, with the exception of higher kidney wt in the $6.6g/m^3$ males ($p<0.05$). There were no significant differences in lung wt at the 24hr or 14-day sacrifices. There were prominent intracytoplasmic eosinophilic inclusions in the renal cortical tubules of males sacrificed at 24hr. No treatment-related effects were seen in the respiratory tract at 24hr or 14-day sacrificed rats. No other significant test article-related microscopic effects were found.
Conclusions	
(study author)	LC_{50} was not reached at $6.6g/m^3$ No deaths occurred during the study. Clinical signs were indicative of the presence of an irritant. The only biologically significant finding

	was microscopic eosinophilic inclusions in the renal cortical tubules of high dose males sacrificed 24hr after exposure.
Data Quality	
Reliability	**1.** Reliable without restrictions.
References	Rose, P.H., Jackson, G.C., Clark, G.C., and Gopinath, C. 1983. Acute inhalation toxicity in rats, 4-hour exposure. Report #MOB 9/83503. Huntingdon Research Center plc, Huntingdon, England, for Mobil Oil Corp., Princeton, NJ Roy, T.A. 1983. Analysis of rerun tower bottoms. Study #1271-81B,C. Mobil Oil Corp, Princeton, NJ
Other	
Last changed	12/18/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Acute Toxicity

Test Substance	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+
Method	
Method/guideline followed	None specified, comparable to standard studies
Type (test type)	Acute
GLP	Not specified
Year	1977
Species/Strain	Rabbits, New Zealand White
Sex	Not specified
No. of animals per sex per dose	10 rabbits/dose
Vehicle	None
Route of administration	Dermal
Test Conditions	Ten New Zealand White rabbits were clipped free of abdominal hair and 6-10 epidermal abrasions were made longitudinally every 2-3cm over the exposed area. The abrasions were sufficiently deep to penetrate the stratum corneum but not deep enough to produce bleeding. A single 5.0g/kg dose was applied to the exposed area, which was then covered with gauze, and the trunk was wrapped with impervious material for 24 hrs. At 24 hr, the rabbits were cleansed and dermal reactions were evaluated by the Draize technique. Rabbits were then observed for 14 days.
Results	
LC_{50} with confidence limits.	One rabbit died on day 8 of the study (dosing was on day 0). All erythema and edema scores were between 1 and 2.
Remarks	
Conclusions	
(study author)	The test material was considered nontoxic by the dermal route.
Data Quality	
Reliability	**1.** Reliable without restrictions. .
References	Moreno, O.M., 1977. Acute dermal toxicity in rabbits. Project #MB77-1855, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp., Paulsboro, NJ Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ
Other	
Last changed	12/18/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Genetic Toxicity - in Vitro

Test Substance	
Test substance	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+
Method	
Method/guideline followed	Standard method per Ames et al, 1975
Type	Reverse mutation bacterial assay
System of testing	Salmonella typhimurium with and without metabolic activation, Sacchromyces cerevisiae
GLP	Yes
Year	1977
Species/Strain	S. typhimurium TA1535, TA1537, TA1538, TA100, TA98; S. cerevisiae D4
Metabolic activation	Yes
Species and cell type	Sprague Dawley adult male rat liver (S9 fraction)
Quantity	50µl S9 fraction in 0.5ml S9 mix/plate
Induced or not induced	Aroclor 1254 induced, administered to male rats 5 days prior to sacrifice
Concentrations tested	0, 0.001, 0.01, 0.10, 1.0 and 5.0µl/plate ± S9; diluted in dimethyl sulfoxide (DMSO); negative control 50 µl DMSO/plate
Statistical Methods	None specified. Positive response criteria per Ames et al., (1975) were number of mutant colonies equal to or greater than 2 –3 times reversion frequency of negative control for each strain. Results must be reproducible in a repeat assay.
Remarks for Test Conditions	Salmonella strains or S. cerevisiae D4 (approx. 10^8/ml) were added to separate test tubes containing 2.0ml molten top agar and appropriate doses of RTB were administered to each tube. Just prior to pouring, 0.5ml S-9 mix was added to tubes receiving metabolic activation and contents was poured on minimal agar plates. Number of tubes or plates ±S9 was not reported. Plates were incubated for 48 hrs at 37^0C and scored for number of colonies on each plate. One complete assay was performed with all strains. Repeat assays of D4 -S9, and TA1538, TA 98+S9 were performed to evaluate results from the 1st assay. Positive control compounds were: 2-nitrofluorene (NF, 100µg/plate) for TA98, TA1538 –S9; methylnitrosoguanidine (MNNG, 10µg/plate) for TA1535, TA100, D4 -S9; quinacrine mustard (QM, 10µg/plate) for TA1537 –S9 and 2-anthramine (ANTH, 100µg/plate) for TA1535, TA100 +S9, dimethylnitrosamine (DMNA, 100µM/plate) for D4 +S9, 2-acetylaminofluorene (AAF, 100µg/plate) for TA1538, TA98 +S9, and 8-aminoquinaline (AMQ, 100µg/plate) for TA1537+S9.
Results	
Genotoxic effects	Rerun tower bottoms was toxic to Salmonella strains TA1535, TA1537, TA98 and S. cerevisiae D4 at 5.0µl/plate. Test material did not increase revertant frequency in any Salmonella strain without metabolic activation. Test of D4 was repeated at doses of 1.0 and 5.0µl/plate due to slight increased revertant frequency at 1.0µl/plate in the 1st test and toxicity at 5.0µl/plate. No increase in revertants was observed at 1.0µl and toxicity persisted at 5.0µl in the repeat assay. In the presence of S9 metabolic activation, no increase in revertant frequency was seen in TA1535, TA1537, TA100 and D4 in the first assay. A repeat test with TA98 and TA100 was performed at 1.0 and 5.0µl/plate because these strains exhibited a dose-related increase in revertant frequency [TA98+ S9: incidence of revertants was 25, 22, 33; TA1538+ S9, incidence of revertants was 26, 48, 88 at negative control, 1.0 and 5.0µl/plate, respectively]. Repeat assay was considered negative [TA98+ S9: incidence of revertants was 55, 57, 99; TA1538+ S9, incidence of revertants was 27, 44, 33 at negative control, 1.0 and 5.0µl/plate, respectively]
Conclusions (contractor)	Rerun tower bottoms did not demonstrate reproducible mutagenic activity in any of the assays and was not considered to be mutagenic under these test conditions.

Data Quality *Reliabilities*	**2.** Reliable with restrictions. Report did not indicate number of tubes or plates used per dose. Criteria for a positive response were not defined in the report.
Reference	Brusick, D.J. 1977. Mutagenicity evaluation of MCTR-36-77. LBI Proj #2683. Litton Bionetics, Inc. Kensington, MD for Mobil Chemical Co., Edison, NJ. Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ Ames, B.N., McCann, J., Yamasaki, E. 1975. Mutat. Res. 31: 347-364
Other *Last changed*	12/18/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Genetic Toxicity - in Vitro

Test Substance	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+
Test substance	
Method	
Method/guideline followed	Standard method, no guideline specified
Type	Sister chromatid exchange (SCE)
System of testing	Human lymphocytes
GLP	Yes
Year	1981
Species/Strain	Human venous blood, primary culture
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	1st trial : 0, 0.0003, 0.001, 0.003, 0.011, 0.033, 0.111, 0.333, 1.1, and 3.3µl/ml 2nd trial: 0. 0.02, 0.04, 0.06, 0.08, and 0.10µl/ml, diluted in dimethyl sulfoxide (DMSO). Negative control was 1% DMSO (100µl/culture).
Statistical Method	Students t-test to compare SCE frequency in treated cultures with negative controls. Positive results require approximate doubling in SCE frequency over background levels at a minimum of 3 doses. In the absence of doubling, positive response requires a statistically significant increase at a minimum of 3 doses and a positive dose response.
Remarks for Test Conditions	Human venous blood was drawn into a sterile heparinized syringe; cultures were initiated by adding 0.6ml blood to 9.4ml RPMI 1640 medium supplemented with fetal calf serum, 1% antibiotics and 5-bromodeoxyuridine (BrdU, 25µM), and incubated at 37^0C in the dark for 24 hours. Dilutions of test material, DMSO or positive control compound, ethyl methane sulfonate (EMS, 0.1µl/ml) were added to cultures (2 cultures/ dose) and incubation continued for 46-50hrs. Colcemid was added 2-3hrs, prior to harvest, to arrest dividing lymphocytes in metaphase. After growing for 2 cell cycles in the presence of BrdU, chromosomes were fixed and stained using fluorescence (5µg/ml Hoechst 33258) with exposure to black light (15-20 min) and 5% Giemsa for an additional 10-20 min, so that one chromatid is dark and its pair or "sister" is pale, allowing exchanged segments between them to be clearly visible. Fifty cells in M2 (2nd division metaphase)/dose were scored for frequency of SCE/cell and per chromosome. All slides were coded prior to scoring and scored "blind"
Results	
Genotoxic effects	In the first trial, no scorable metaphases were found at 3.3µl/ml; at 1.11µl/ml, 50 metaphases could not be found due to cell cycle delay and a reduction in mitotic index. However, there was a statistically significant increase in SCE at 1.11µl/ml and 0.33µl/ml (13.8 and 11.1 SCE/cell respectively, compared to 7.0 SCE/cell in DMSO controls). In the 2nd trial, cultures were treated at concentrations of 0.02-0.10µl/ml and incubated for an additional 4 hours to allow cells to reach second metaphase. Pronounced cell cycle delay occurred at 0.08 and 0.10µl/ml. There were statistically significant increases in SCE/cell at 0.06 (15.1 SCE/cell), 0.08 (16.1 SCE/cell) and 0.10µl/ml (13.1 SCE/cell) compared to DMSO negative control (10.6 SCE/cell) with some evidence of a dose response. Positive control compound. EMS, induced approximately 33 SCE/cell in both assays. Doubling of SCE incidence over DMSO controls was not reached. While the SCE increase was not large, it was apparently dose related and the authors considered this effect to be a weak positive response.
Conclusions (contractor)	A small but statistically significant increase in sister chromatid exchange was demonstrated in human lymphocytes treated with RTB. Rerun tower bottoms was considered to show a weakly positive response under conditions of this assay.
Data Quality	

Reliabilities	**1.** Reliable without restrictions
Reference	Galloway, S.M. 1981. Mutagenicity evaluation of 081088003 in the sister chromatid exchange assay in human lymphocytes. Assay #5634. Litton Bionetics, Inc., Kensington, MD for Mobil Oil Corp, (Study # 1711-80) Princeon, NJ Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ
Other	
Last changed	12/18/01 (Prepared by a contractor to the Olefins Panel)

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Repeated Dose Toxicity

Test Substance	Rerun tower bottoms (RTB, 1271-81). Brown liquid. 140^0F (60^0C) vapor: 35%
Remarks	alkanes/alkenes, 60-65% benzene derivatives, and 2-3% naphthalenes. Benzene, toluene and styrene were 53% of vapor.
Method	
Method/guideline followed	Standard method, no guidelines specified.
Test type	Sub-acute toxicity from a Combined 10 day inhalation toxicity and teratology range-finding study
GLP	Yes
Year	1984
Species	Rat
Strain	Sprague Dawley [COBS:CD (SD)BR] from Charles River, UK
Route of administration	Whole body inhalation
Duration of test	12 days
Doses/concentration levels	0, 0.15, 0.74 and 5.1g/m^3 actual analyzed concentration
Sex	Males and females (10/sex/group)
Exposure period	10 consecutive days
Frequency of treatment	6 hr/day
Control group and treatment	10 males, 10 females exposed to room air, 6hr/day for 10 days
Post exposure observation period	None
Statistical methods	Bartlett's test for heterogeneity of variance. Body wt., food and water consumption employed analysis of variance (ANOVA). Hematology and blood chemistry used ANOVA, William's test. For organ wts, where Bartlett's test was significant at 1%, log transformation was used to see if heterogeneity was removed. Analysis of covariance or ANOVA followed by William's test was then used for significance.
Test Conditions	Male and female rats (approx 13 wks old; males 240-260g, non-pregnant females 180-200g) were housed 5/sex/cage in polypropylene cages with removable mesh tops and floors, on racks within individually ventilated areas between treatment periods to prevent passive exposure to RTB vapors from rats in other dose groups. Rooms were maintained at temperature range of 20-22^0C, relative humidity 60-64% and a 12 hr light/dark cycle. Rat chow and tap water were available ad lib except during exposure. Rats were assigned to groups by body weight using a pseudo-random computer program. Exposure vapors were continuously generated from a supply of neat oil at 140^0F in a double-surface glass condenser. Nitrogen gas carrier was passed through the condenser, diluted with air, and metered into the exposure chambers. Generation conditions at each dose level were designed to produce approximately 20% vaporization of RTB. Total chamber airflow was set for 12 air changes/hr. Chamber vapor concentrations were monitored by an infrared gas analyzer, and samples were collected every hour on carbon sorption tubes for analysis by gas chromatography/ flame ionization detection. Target concentrations were 0, 0.1, 0.75 and 5.0g/m^3 (actual 0, 0.15, 0.74 and 5.1g/m^3) determined from an acute inhalation study. During exposure, rats were housed in stainless steel mesh cages, partitioned to isolate each rat, set on supports in the inhalation chamber. Avg. chamber atmospheres were 23.5^0F, 41-59% relative humidity. Positions of animals were alternated daily. After daily exposure was completed, chambers were allowed to clear for 30 min before the rats were removed and returned to holding cages. Animals were observed twice daily for clinical signs, before and after exposure as animals were moved between cages. Animals were weighed 7 days prior to exposure and on day 1 (time 0 of exposure), 3, 7, and 10, before daily exposure began. Daily food and water consumption was recorded by cage, beginning 7 days prior to start of exposure. Blood was drawn on day 11 (the day following the final exposure) from all animals (fasted 16hr overnight). Evaluated hematology parameters were hemoglobin, red cell (RBC) count, total white cell (WBC) count and differential WBC counts, and packed cell volume (PVC); blood chemistry parameters were plasma glucose, serum total protein, serum albumin, albumin/lobulin (A/G) ratio, urea nitrogen, alkaline phosphatase, and serum glutamic-pyruvic transaminase (GPT). On day 11, all rats were killed: lungs/trachea, liver, kidneys, spleen, thymus, adrenal and gonads were weighed; 34 tissues

	were preserved for possible microscopic examination. Detailed microscopic examination was performed on hematoxylin-eosin stained sections of liver, kidney, testes/ovary, trachea, lung and head from all rats; thymus, spleen and adrenal from control and 5.1g/m^3 groups (10M, 10F) and spleen from 10 males only in the 0.74g/m^3 group.
Results NOAEL (NOEL) LOAEL (LOEL) Remarks	NOAEL was not established LOAEL both sexes = 0.15g/m^3 based on statistically significant dose-related changes in kidney wt and pathology for all males, decreased urea nitrogen levels in all females (Assigned by reviewer). No deaths occurred during the study. Clinical signs during exposure observed in the 5.1g/m^3 group were abnormal body posture, closing or partial closing of eyes on every day of exposure consistent with exposure to an irritant atmosphere. Following exposure, some 5.1g/m^3 animals exhibited lethargy, red/brown staining around head or snout, urine staining, salivation, occasional ataxia and lacrimation, peripheral vasodilation mainly on the first few days of exposure, and hair loss in a few rats at the end of the 10-day treatment period. Increased urination overnight was noted in these high dose rats (both sexes). Lower dose groups showed few clinical signs. Body wt and weight gain over 10 days were similar to controls for all animals in 0.15 and 0.74g/m^3 groups (male gain: 39, 40, 40 g; female gain: 12, 13, 14g in control, 0.15 and 0.74g/m^3 groups, respectively. At 5.1 g/m^3, males failed to gain weight (-2g), resulting in a statistically significantly difference compared to controls; females gained less (8g) than controls but effect was not statistically significant. Overall, food consumption for 10 days was statistically significantly lower ($p<0.01$) in 5.1g/m^3 males (202g) and females (149g) compared to controls (males, 248g, females, 168g), however female's food consumption became comparable to controls between days 6-9 of exposure. Other groups were similar to controls throughout. High dose rats of both sexes, particularly high dose females, consumed statistically significantly ($p<0.01$) more water than controls (males, 537g; females, 576g compared to control males, 324g; females 217g); all other groups were comparable to controls. Hematology parameters: Males exposed to 5.1g/m^3 exhibited statistically significantly higher values for PCV, Hb, and RBC than controls, however the absolute values for individual animals were within historical control ranges for the laboratory; 5.1g/m^3 females And other treated groups were comparable to controls. Blood chemistry parameters: the 5.1g/m^3 males had significantly higher protein, albumin, and GPT levels and significantly lower alkaline phosphatase level than controls; in females the A/G ratio and GPT levels were higher than controls. The A/G ratio in 0.74g/m^3 females was also higher than controls. Urea nitrogen levels in females at all dose levels were statistically significantly lower than controls (21, 17, 19, and 14mg/dl in control, 0.15, 0.74 and 5.1g/m^3, respectively). Again, absolute values for individual rats for these parameters were within acceptable ranges for age and strain of rat. At necropsy, increased incidence of alopecia and incidence of hemorrhagic areas in the mucosa of stomachs in 5.1g/m^3 female rats was observed macroscopically. Liver, kidney and adrenal wt tended to be higher than controls for most groups in a dose-related fashion; spleen and thymus wt in 0.74g/m^3 and 5.1g/m^3 groups tended to be less than controls. Differences were statistically significant for males at all dose levels for kidney wt, at 0.74 and 5.1g/m^3 for liver (increase) and spleen (decrease), and at 5.1g/m^3 only for adrenal (increase) and thymus (decrease). In females, changes were statistically significant only at 5.1g/m^3. Microscopic pathology results in the 5.1g/m^3 group showed enlargement of centrilobular hepatocytes in liver (5/10 males, 8/10 females), decreased cellularity of red pulp (6/10ma les) in spleen, minimal involution of thymus (3/10 males, 3/10 females), minimal increase in fine vacuolation of zona fasciculata (6/10 males, 4/10 females), and minimal increase in cortical width (7/10 females, only) in adrenals. Male rats from all dose groups has eosinophilic intracytoplasmic inclusions in renal cortical tubules in the kidney. Other changes at 5.1g/m^3 or in other dose groups were considered spontaneous in origin and of no toxicological significance.
Conclusions (study authors)	NOAEL was not established. Decreases in body wt gain and food intake, increases in water intake, changes in hematology, blood chemistry and organ wts, and pathological findings were induced by rerun tower bottoms in rats exposed for 10 days. RTB appeared to induce diuretic action with comp ensatory water intake in both sexes. Incomplete compensation in male rats resulted in minimal hemoconcentration (increased PCV, Hb, RBC and total protein

	at 5.1g/m^3); in female rats, a decrease in urea nitrogen (all doses) resulting from increased urine flow. Direct effect on the liver was demonstrated by elevated GPT levels, and enlargement of centrilobular hepatocytes in both sexes at the high dose. The occurrence of intracytoplasmic inclusions in renal cortical tubules was observed in male rats in all groups, increasing in severity with dose. [This effect could later be correlated with characteristic hydrocarbon-induced nephropathy seen in male rats. Reviewer's comment]. Changes in adrenals, spleen and thymus probably correlate with stress response and adaptation to exposure to Rerun tower bottoms.
Quality Reliabilities	**1.** Reliable without restrictions
References	Rose, P.H., Street, A.E., James, P. and Gopinath, C. 1984. Compound 1271-81 vapor ten-day inhalation toxicity and teratology range-finding study in rats. HLS-MOB7/83656. Huntingdon Research Centre, plc. Cambridgeshire, England for Mobil Oil Corp, Princeton, NJ (see separate summary for teratology data). Rose, P.H., Jackson, G.C., Clark, G.C., and Gopinath, C. 1983. Acute inhalation toxicity in rats, 4-hour exposure. Report #MOB 9/83503. Huntingdon Research Center plc, Huntingdon, England, for Mobil Oil Corp., Princeton, NJ Roy, T.A. 1983. Analysis of rerun tower bottoms. Study #1271-81B,C. Mobil Oil Corp, Princeton, NJ
Other Last changed	12/18/01 (Prepared by contractor to Olefins Panel)

Developmental Toxicity/Teratogenicity

Test Substance	Rerun tower bottoms (RTB, 1271-81). Brown liquid. 140^0F (60^0C) vapor: 35% alkanes/alkenes, 60-65% benzene derivatives, and 2-3% naphthalenes. Benzene, toluene and styrene were 53% of vapor.
Remarks	
Method	
Method/guideline followed	Standard method, no guidelines specified.
Test type	Teratology range-finding from a Combined 10 day inhalation toxicity and teratology range-finding study
GLP	Yes
Year	1984
Species	Rat
Strain	Sprague Dawley [COBS:CD (SD)BR] from Charles River, UK
Route of administration	Whole body inhalation
Concentration levels	0, 0.15, 0.74 and 5.1g/m^3 actual analyzed concentration
Sex	Time-mated females (5/ group)
Exposure period	Day 6-15 of gestation
Frequency of treatment	6 hr/day
Control group and treatment	5 time mated females, exposed to room air 6hr/day from day 6-15 of gestation
Duration of test	20 days
Statistical methods	None performed. Small sample size in range-finding study
Remarks for Test Conditions.	Twenty sexually mature time mated female rats (171-200g at study initiation) were assigned to one of four groups (5/group) based on body wt on day 1 of gestation. The day of mating identified by presence of a vaginal plug =day 0 of gestation. Rats were housed 5/group in polypropylene cages with stainless steel mesh floors and tops when not in exposure chambers. Animal rooms were maintained at 20-22^0C temperature, 60-64% relative humidity and a 12hr light/dark cycle. Rat chow and tap water were available ad lib except during exposure. Exposure vapors were continuously generated from a supply of neat oil at 140^0F in a double-surface glass condenser. Nitrogen gas carrier was passed through the condenser, diluted with air, and metered into the exposure chambers. Generation conditions at each dose level were designed to produce approximately 20% vaporization of RTB. Total chamber airflow was set for 12-air changes/hr. Chamber vapor concentrations were monitored by an infrared gas analyzer, and samples were collected every hour on carbon sorption tubes for analysis by gas chromatography/ flame ionization detection. Target concentrations were 0, 0.1, 0.75 and 5.0g/m^3 (actual 0, 0.15, 0.74 and 5.1g/m^3) determined from an acute inhalation study. During exposure, rats were housed in stainless steel mesh cages, partitioned to isolate each rat, set on supports in the inhalation chamber. Avg. chamber atmospheres were 23.5^0F, 41-59% relative humidity. Positions of animals were alternated daily. After daily exposure was completed, chambers were allowed to clear for 30 min before the rats were removed and returned to holding cages. Animals were observed twice daily for clinical signs, before and after exposure, and from post-treatment days 16-20 of gestation. Rats were weighed on day 1, 3, 6, 10, 14, 17 and 20 of gestation. Food and water consumption were recorded daily by cage. On day 20 of gestation, rats were killed by CO2 asphyxiation, dissected and exa mined for congenital abnormalities and macroscopic pathological changes in maternal organs. Ovaries and uteri were examined for number of corpora lutea, number and distribution of live fetuses, embryonic/fetal death, fetal abnormalities and individual live fetal wt from which litter wt was calculated. Uteri or individual uterine horns without visible implantations were immersed in 10% ammonium sulfide to evaluate pregnancy. Live fetuses were examined externally and weighed. One half of fetuses in each litter were preserved in Bouin's fixative for soft tissue evaluation by free-hand sectioning if required, one half were fixed, sexed internally, eviscerated, cleared and stained with Alizarin Red for skeletal examination if required. Group mean values for litter size, embryonic death, and pre- and post-implantation loss were calculated.
Results	
NOAEL maternal toxicity	NOAELmaternal = 0.74g/m3 (Assigned by reviewer)

NOAEL developmental toxicity	NOAEL developmental = 0.15g/ m^3 (Assigned by reviewer)
Maternal effects	During exposure, 5.1g/m^3 females only showed closing or partial closing of eyes, inactivity and abnormal body posture. Between exposures, 5.1g/m^3 females showed lethargy, red staining of snout, slight general vasodilation, and increased urination and staining of urogenital region with occasional signs of slight ataxia and increased salivation. At 0.74g/m^3, one rat had red staining on snout on 2 occasions. At 5.1g/m^3, food consumption was reduced during the treatment period and water consumption was markedly increased during and post-treatment; other groups were similar to controls. At 5.1g/m^3, body wt loss occurred during the first four days of treatment (-1.0g compared to 21.2, 25.0 and 19.3g in control 0.15,and 0.74g/m^3 groups, respectively); body wt gain in the 0.74g/m^3 group was marginally lower than controls, but regained parity by day 14. From days 14-20 of gestation, high dose weight gain improved but did not reach parity with other dose groups. At necropsy, no gross abnormalities were observed in parental animals. Pregnancy rate was 80-100% in all groups, incidence of corpora lutea, implantations, and live young were comparable to or higher in treated groups than controls.
Embryo/fetal effects	At 5.1g/m^3, mean fetal wt was markedly reduced (2.97g compared to 3.57g in controls) and mean number of intra-uterine deaths was higher (1.6 compared to 0.6 in controls); these effects of lesser magnitude were also observed in the 0.74g/m^3 group litters. No fetal malformations were observed at necropsy.
Conclusions (study authors)	Rerun tower bottoms induced clinical signs of exposure in pregnant rats, principally at the highest dose level, 5.1g/m^3, and marginal body wt loss and decreased food consumption during the early days of exposure; increased water consumption was observed throughout exposure and the post-treatment period. Mean fetal wt was reduced and mean number of intra-uterine deaths was increased at 5.1g/m^3. No teratogenic effects (malformations or variations) were observed in offspring at necropsy. The doses employed in this assay would be acceptable for a definitive teratology study of Rerun tower bottoms.
Data Quality *Reliabilities*	**1.** Reliable without restrictions
References	Rose, P.H., Street, A.E., James, P. and Gopinath, C. 1984. Compound 1271-81 vapor ten-day inhalation toxicity and teratology range-finding study in rats. HLS-MOB7/83656. Huntingdon Research Centre, plc. Cambridgeshire, England for Mobil Oil Corp, Princeton, NJ (see separate summary for toxicity data). Rose, P.H., Jackson, G.C., Clark, G.C., and Gopinath, C. 1983. Acute inhalation toxicity in rats, 4-hour exposure. Report #MOB 9/83503. Huntingdon Research Center plc, Huntingdon, England, for Mobil Oil Corp., Princeton, NJ Roy, T.A. 1983. Analysis of rerun tower bottoms. Study #1271-81B,C. Mobil Oil Corp, Princeton, NJ
Other Last *changed*	12/18/01 (Prepared by contractor to Olefins Panel)

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Developmental Toxicity/Teratogenicity

Test Substance	
Remarks	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+
Method	
Method/guideline followed	Standard method, no guidelines specified.
Test type	Teratology study
GLP	Yes
Year	1981
Species	Rabbit
Strain	New Zealand White
Route of administration	Dermal
Concentration levels	0, 62.5, 125, 250, 500 and 1000mg/kg/day
Sex	Females (5/ group)
Exposure period	Day 6-18 of gestation
Frequency of treatment	Once daily
Control group and treatment	5 females/group, distilled water applied dermally from day 6-18of gestation
Duration of test	29 days
Statistical methods	None performed. Small sample size in range-finding study
Remarks for Test Conditions.	Thirty sexually mature, virgin female rabbits (8.5-9.5 months old at time of insemination), had been acclimated for 30 days, ear-tagged with a unique number for identification, and assigned by body wt to one of 5 groups (5 females/group). All rabbits were individually housed in suspended wire mesh cages and maintained in a temperature and humidity controlled room (specifics not given) with a 12hr light/dark cycle. Rabbit chow and tap water was available ad lib. During the treatment period, all animals were maintained in a specially ventilated room under identical housing conditions. Fema les were artificially inseminated with semen from one of 3 proven male rabbits of same strain and source. Semen was collected using an artificial vagina; only semen with 50% or greater motility was used. Ejaculate was diluted with 4.0ml of 0.9% NaCl, USP injectable grade at 35^0C and 0.25-0.5ml was introduced into the anterior vagina. Within one hour after insemination, ovulation was induced by injection of 100units of chorionic gonadotropin into the marginal ear vein. Day of insemination =day 0 of gestation. Semen from one male was used to inseminate an equal number of females in each group. Prior to test article administration (gestation day 6) and as often as necessary thereafter, the back of each female was shaved and each animal was fitted with a Saf-T Shield® collar. Rerun tower bottoms was applied undiluted to the intrascapular area in a single daily application on gestations days 6-18. The site was uncovered, non-abraded and was not washed. Control rabbits were treated with distilled water at a concentration of 1.08ml/kg in a comparable regimen. Dosages were determined from individual body wt at day 6; collars were removed on gestations day 19. Prior to, during treatment, and until day 29 of gestation, females were observed for mortality, overt changes in appearance and behavior, and clinical signs of toxicity (day 6-29 of gestation). Females showing signs of abortion remained on study until scheduled sacrifice to determine any test article related effect; aborted tissue was discarded. Gross necropsy was performed on all rabbits not surviving to scheduled necropsy. Maternal body wt were recorded on gestation days 0, 6, 12, 18, 24 and 29. Dams were sacrificed on day 29, uteri were excised and the location of viable and non-viable fetuses, early and late resorptions, number of total implantations and corpora lutea were recorded. Abdominal and thoracic cavities and organs of dams were examined for gross evidence of morphological changes and discarded. Uteri from apparent non-gravid females were opened and placed in 10% ammonium sulfide solution for confirmation of pregnancy status.
Results	
NOAEL maternal toxicity	NOAEL maternal was not established (Rabbits at lowest dose aborted)
NOAEL developmental toxicity	NOAEL developmental was not established. (Reviewer's comments).

Maternal effects	All animals in control, 62.5, 125 and 250mg/kg groups survived to the end of the study. One 500mg/kg female aborted and died on gestation day 229, and 3 died in the 1000mg/kg day group – one on day 21, 2 on day 27 of gestation (1 aborted prior to death). Prior to death, matting and/or staining of hair in the urogenital region and lack of coordination of the hind limbs was noted. At necropsy, reddened foci and/or focal erosion of stomach lining was observed, suggesting that, despite collars, inherent preening activity and subsequent oral ingestion of RTB occurred. Two females that died in the 1000mg/kg group had tan, creamy intestinal contents; distention of gall bladder was also noted in one of these rabbits. In the 1000mg/kg rabbit that died on day 21 of gestation, the left uterine horn was 1/3 as long as the right horn with thickening of the endometrium. Eleven rabbits aborted: 2 each in control, 62.5 and 500mg/kg/day groups, 3 in 125mg/kg group and 1 each in the 250 and 1000mg/kg groups. Since 40% (2/5) control rabbits aborted, inhalation of RTB may be a contributing factor. Dermal irritation at application site, erythema, edema, fissuring, was observed in all treated rabbits, first reported on day 13 of gestation and persisting until sacrifice. Eschar formation was reported later in the treatment period (days not given) and persisted until sacrifice. Nasal discharge, soft stool, occasional instances of dry, yellow matter in nasal region, hair loss, and white ocular discharge were noted in controls and treated animals with similar frequency. At necropsy, one female in the 62.5mg/kg group had a 2cm diameter abscess on the ventral neck; one 125mg/kg female had several erosions in the lining of the gall bladder, and one female had slight pitting of kidneys; one 500mg/kg female had accentuated lobulation of the liver. Fluid in the abdominal cavity was observed in one 62.5mg/kg female and one 500mg/kg female. Mean maternal body wt losses occurred in all treated groups during the treatment period (day 6-18 of gestation). Mean body wt gains were seen following the end of exposure (day 18-29 0f gestation) in the 62.5, 125 and 500mg/kg groups; mean gains exceeded controls. Continued wt loss occurred in the 250 and 1000mg/kg groups post-treatment. Post-implantation loss occurred in the control group and in treated groups below or within laboratory historical control values (0.4-1.6); losses did not occur in a dose-related pattern. No biologically meaningful differences were observed in mean number of corpora lutea, total implantations or viable fetuses in any treatment group compared to controls. Decrease in mean number of total implants correspond to decrease in mean number of viable fetuses in 125 (one dam), 250 and 500mg/kg/day groups- one dam in each group had only early resorptions. Mean number of total implants and viable fetuses in 1000mg/kg group was greater than control mean values (9.5 implants and 8.5 fetuses in 1000mg/kg group compared to 6.7 implants and 6.7 fetuses in controls).
Embryo/fetal effects	No grossly observed teratogenic effects were reported.
Conclusions (study authors)	Dermal irritation, erythema, edema, fissuring and eschar formation, were induced by Rerun tower bottoms in all treated groups. Mean maternal body wt losses occurred during treatment (day 6-18 of gestation). Four rabbits died (1 in 500mg/kg, 4 in 1000mg/kg group); 2 of which aborted prior to death. Eleven rabbits aborted, including 2 in control group, suggesting that inhalation of RTB may have been a contributing factor. Due to high abortion rate in every treatment group, a dose of 62.5mg/kg/day would be excessive for definitive teratology study.
Data Quality *Reliabilities*	**1.** Reliable without restrictions
References	Spicer, E.J.F and Schardein, J.L. 1981. Pilot dermal teratology study in rabbits (MCTR-169-79). IRDC Proj. #450-013. International Research and Development Corp., Mattawan, Mich. for Mobil Oil Corp., Princeton, NJ Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ
Other *Last changed*	12/18/01 (Prepared by contractor to Olefins Panel)

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Developmental Toxicity/Teratogenicity

Test Substance	
Remarks	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+
Method	
Method/guideline followed	Standard method, no guidelines specified.
Test type	Teratology study
GLP	Yes
Year	1981
Species	Rabbit
Strain	New Zealand White
Route of administration	Dermal
Concentration levels	0, 10, 25, and 50mg/kg/day
Sex	Females (16/ group)
Exposure period	Day 6-18 of gestation
Frequency of treatment	Once daily
Control group and treatment	Proximate and remote control groups: 16 females/group, distilled water applied dermally from day 6-18of gestation
Duration of test	29 days
Statistical methods	All analyses compared proximate to remote control and each control group to each treatment group ($p<0.05$). Chi square with Yates correction and/or Fisher's exact probability test used for male to female sex distribution and number of litters with malformations. Mann-Whitney U-test used for number of early and late resorptions and post-implantation losses. One-way analysis of variance, Bartlett's test, appropriate t-test for equal or unequal variance and Dunnett's multiple comparison tables were used for other fetal parameters and mean fetal body wt.
Remarks for Test Conditions.	Eighty sexually mature, virgin female rabbits (approx. 7 months old at time of insemination), had been acclimated for 63-68 days, ear-tagged with a unique number for identification, and assigned by body wt to one of 3 groups (16 females/group). All rabbits were individually housed in suspended wire mesh cages and maintained in a temperature and humidity controlled room (specifics not given) with a 12hr light/dark cycle. Rabbit chow and tap water was available ad lib. During the treatment period, all animals were maintained in a specially ventilated room under identical housing conditions. Remote control females were housed separately. Females were artificially inseminated with semen from one of 10 proven male rabbits of same strain and source. Semen was collected using an artificial vagina; only semen with 50% or greater motility was used. Ejaculate was diluted with 4.0ml of 0.9% NaCl, USP injectable grade at 35^0C and 0.25-0.5ml was introduced into the anterior vagina. Within one hour after insemination, ovulation was induced by injection of 100units of chorionic gonadotropin into the marginal ear vein. Day of insemination =day 0 of gestation. Procedures were performed on 2 consecutive days and equal numbers of females were inseminated in each group/day. Prior to test article administration (gestation day 6) and as often as necessary thereafter, the back of each female was shaved and each animal was fitted with a Saf-T Shield® collar. Doses were selected following a pilot range-finding study. Rerun tower bottoms was applied undiluted to the intrascapular area in a single daily application on gestations days 6-18. The site was uncovered, non-abraded and was not washed. Control rabbits were treated with distilled water in a comparable regimen. Dosages were determined from individual body wt at day 6; collars were removed on gestations day 19-20. Prior to, during treatment, and until day 29 of gestation, females were observed for mortality, overt changes in appearance and behavior, and clinical signs of toxicity (day 6-29 of gestation). Females showing signs of abortion were sacrificed and examined for grossly evident morphological changes; intact fetuses were examined externally and preserved in formalin. Gross necropsy was performed on all rabbits not surviving to scheduled necropsy, and tissue was saved in formalin, if appropriate. Maternal body wt were recorded on gestation days 0, 6, 12, 18, 24 and 29. Due to a weighing error on

	gestation day 18, body wt from 8 females in the 25mg/kg group were not included in calculations of group mean. Dams were sacrificed on day 29, uteri were excised and weighed prior to removal of fetuses. The location of viable and non-viable fetuses, early and late resorptions, number of total implantations and corpora lutea were recorded. Abdominal and thoracic cavities and organs of dams were examined for gross evidence of morphological changes and discarded. Uteri from apparent non-gravid females were opened and placed in 10% ammonium sulfide solution for confirmation of pregnancy status. All fetuses were individually weighed and examined for external malformations and variations. Each was internally sexed and examined for visceral malformations and variations including brain by mid-coronal slice, and heart dissection by modification of Staples' technique. Eviscerated skinned fetuses were individually numbered and tagged, fixed in alcohol, macerated in KOH and stained with Alizarin red S for skeletal examination.
Results	
NOAEL maternal toxicity	NOAEL maternal was not established (Dermal irritation at all levels)
NOAEL developmental toxicity	NOAEL developmental = 50mg/kg/day (Levels assigned by reviewer).
Maternal effects	All animals in control, 10, and 25mg/kg groups survived to the end of the study. One 50mg/kg non-gravid female died on gestation day 11, exhibiting edematous lungs and hyperemic tracheal mucosa at necropsy, suggesting respiratory distress a s the case of death. There were 14/16 apparently gravid rabbits in the proximate control, 10/16 in the remote control, 14/16, 12/16 and 14/16 in the 10, 25 and 50mg/kg groups, respectively. Eight rabbits aborted and were sacrificed; one each in proximate control (gestation day 23) and 25 mg/kg group (gestation day 26), 2 in 10mg/kg group (gestation day 26), and 4 in 50mg/kg group (0ne each on gestation day 20, 22,27, 28). Majority of rabbits that aborted in the 50mg/kg group had congested lungs or foci on lungs at necropsy. Dermal irritation at application site was observed in all treatment groups. Redness and swelling of application site was evident at initiation of treatment and persisted throughout gestation. Majority of females developed fissuring and flaking midway through treatment that persisted until sacrifice. Peeling of epidermal layer was reported in 7 rabbits, one at 10, 2 at 25 and 4 at 50mg/kg groups for several days during the treatment period. Dermal irritation did not occur in either control group. Soft stool was evident in the majority of rabbits treated with 50mg/kg/day. No treatment related findings were observed in organs of maternal females at necropsy. There were no biologically meaningful differences in mean maternal body wt gain between controls, or between any treated group and remote controls during the treatment period (day 6-18 of gestation)- gains were 147,212, 145 and 148 in remote control, 10, 25 and 50mg/kg/day groups, respectively. Comparable losses in mean adjusted body wt (female body wt on gestation day 29 minus uterus and contents) were observed in both controls and all treated groups [-104, -272, -55, -171, -244 in proximate controls, remote controls, 10, 25 and 50 mg/kg/day, respectively. Since a substantial adjusted body wt loss was observed in remote control animals, these changes were not considered treatment- related.
Embryo/fetal effects	At caesarean section, there were no statistically significant or biologically meaningful differences in mean number of corpora lutea, total implantations, post-implantation loss, early or late resorptions, viable fetuses or fetal sex distribution between controls or in 10, 25 or 50mg/kg/day groups. The incidence of gravid dams with viable fetuses was 100% in all groups: 13, 10, 12, 11, and 10 and viable fetuses/dam were 6.7, 6.7, 5.6, 7.1, and 7.0 in proximate control, remote control, 10, 25, and 50mg/kg/day groups. A decrease in mean number of corpora lutea. A decrease in mean number of corpora lutea and total implantation with corresponding decrease in mean number of viable fetuses was observed in the 10mg/kg day group when compared to remote controls, but effect were not observed at higher doses and were not considered treatment related. A lower mean fetal body wt. was observed at the 50mg/kg/day level (36.4g compared to 40.4g in proximate control and 42.9g in remote controls) but was within the laboratory's historic control range (35.2-42.6g). There were no biologically meaningful or statistically significant differences in number of litters with malformations in either control group or RTB-treated groups. Hydrocephaly was observed in 4 fetuses in one litter in the 10mg/kg group and 3 fetuses in 2 litters (2 in one litter and 1 in one litter) in the 25mg/kg/day group. No hydrocephaly was seen at 50mg/kg/day so these finding were considered not treatment related. Spina bifida occurred in one fetus in one 50mg/kg/day group litter. An increase

	in number of fetuses and number of litters with 13th rudimentary rib(s) occurred in the 50 mg/kg/day group which slightly exceeded the historical control range; % incidence was 25.7% affected fetuses in 80.5% litters compared to 21.8% affected fetuses in 61.5% litters in proximate control and 23.9 affected fetuses in 70% litters in remote control groups. All other genetic and developmental variations were within historical control range.
Conclusions (study authors)	Dermal irritation was observed in all rabbits treated with Rerun tower bottoms with a dose-related increase in number of females exhibiting peeling of the epidermal layer. One 50mg/kg/day non-gravid female died during gestation, probably the result of respiratory distress. Eight rabbits aborted, one each in proximate controls and 25mg/kg group, 2 in 10mg/kg group, 4 in 50 mg/kg group; no abortions occurred in remote control animals. The reason for the high abortion rate maybe consequence of severe dermal irritation in treated groups and inhalation of RTB vapor was a contributing factor. No significant differences in mean maternal or fetal observations at caesarean section or in number of litters with malformations and genetic or developmental variations were identified in RTB-treated groups compared to controls. No fetal toxicity was observed and Rerun tower bottoms did not produce a teratogenic response at any treatment level.
Data Quality *Reliabilities*	**1.** Reliable without restrictions
References	Spicer, E.J.F and Schardein, J.L. 1981. Dermal teratology study in rabbits (MCTR-169-79). IRDC Proj. #450-009. International Research and Development Corp., Mattawan, Mich. for Mobil Oil Corp., Princeton, NJ Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ
Other *Last changed*	12/18/01 (Prepared by contractor to Olefins Panel)